

National Institute
of
Allergy and Infectious
Diseases

Annual Report of Intramural Activities

October 1, 1983-September 30, 1984

U.S. Department of Health and Human Services
Public Health Service
National Institutes of Health



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of
Allergy and Infectious
Diseases

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U.S. Department of Health and Human Services
Public Health Service
National Institutes of Health

For Administrative Use

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1984

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NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

1984 ANNUAL REPORT OF INTRAMURAL ACTIVITIES

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NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

1984 ANNUAL REPORT PROJECT NUMBER LISTING*

Z01 AI

00006-13 LMM
00011-19 LVD
00013-21 LMM
00020-09 LVD
00027-17 LMM
00030-16 LI
00035-09 LI
00036-19 LI
00037-17 LI
00043-19 LCI
00045-16 LCI
00047-15 LCI
00048-14 LCI
00057-11 LCI
00058-10 LCI
00061-22 EB
00063-14 EB
00065-11 LMSF
00071-13 OSD
00072-13 LPVD
00073-19 LPVD
00074-12 LPVD
00082-23 EB
00085-07 LPVD
00086-07 LPVD
00094-25 LPD
00097-26 LPD
00098-28 LPD
00099-14 LPD

Z01 AI

00102-10 LPD
00103-17 LPD
00108-13 LPD
00123-18 LVD
00126-11 LVD
00131-17 LMI
00134-22 LMI
00135-10 LVD
00136-12 LMI
00138-10 LVD
00141-10 LMI
00143-15 LMI
00144-20 LMI
00145-17 LMI
00146-11 LMI
00147-09 LI
00148-09 LI
00153-07 LMI
00154-09 LMI
00155-09 LCI
00161-07 LPD
00162-08 LPD
00166-07 LIG
00169-07 LIG
00170-07 LIG
00171-07 LIG
00172-07 OSD
00173-07 LIG
00180-06 LIG

*Does not include terminated or inactive projects

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

1984 ANNUAL REPORT PROJECT NUMBER LISTING

Z01 AI

00182-06 OSD
00183-06 OSD
00186-11 LMI
00189-05 LCI
00190-06 LMM
00191-06 OSD
00192-06 LCI
00193-05 LMSF
00194-05 LMSF
00197-05 LPD
00199-05 LPVD
00201-05 RMOB
00203-05 LMI
00205-04 LVD
00208-04 LPD
00210-04 LIR
00211-04 LIR
00212-04 LIR
00213-04 LIR
00216-04 LMSF
00218-03 LMM
00219-03 LMM
00222-03 LMM
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00224-03 LI
00225-03 LI
00226-03 LI
00228-03 OSD
00229-03 LI

Z01 AI

00230-03 LMSF
00231-03 LMSF
00232-03 LMSF
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NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

1984 ANNUAL REPORT PROJECT NUMBER LISTING

Z01 AI

00265-03 LPVD
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00269-03 LCI
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Z01 AI

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00334-03 LID
00335-03 LID
00338-03 LID
00339-02 LID

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

1984 ANNUAL REPORT PROJECT NUMBER LISTING

Z01 AI

00340-03 LID
00341-03 LID
00342-03 LID
00343-03 LID
00344-03 LID
00345-03 LID
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00349-02 LI
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00351-02 LPD
00352-02 LIG
00353-02 LMM
00354-02 LCI
00356-02 LCI
00357-02 LCI
00358-02 LCI
00359-02 LCI
00360-02 LCI
00361-02 LCI
00362-02 LMSF
00365-02 LID
00366-02 LID
00367-02 LID
00368-02 LID
00369-02 LID
00370-02 LID
00371-02 LID

Z01 AI

00372-02 LID
00373-02 LID
00376-02 LVD
00379-02 LCI
00383-02 OSD
00384-02 OSD
00385-02 OSD
00386-01 LPVD
00387-01 LPVD
00388-01 LMM
00389-01 LIG
00390-01 LIR
00391-01 LVD
00392-01 LVD
00393-01 LVD
00394-01 LI
00395-01 LMM
00396-01 LCI
00397-01 LCI
00398-01 LCI
00399-01 LMM
00400-01 LI
00401-01 LPVD
00402-01 EB
00403-01 LI
00404-01 LID
00405-01 LID
00406-01 LID
00407-01 LID

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

1984 ANNUAL REPORT PROJECT NUMBER LISTING

Z01 AI

00408-01 LID

00409-01 LID

00410-01 LID

00411-01 LID

00412-01 LMSF

00413-01 LMSF

00414-01 LBV

00415-01 LMM

00416-01 LVD

00417-01 OSD

00418-01 LPVD

00419-01 OSD

00420-01 OSD

00421-01 OSD

00422-01 OSD

00423-01 LMI

00424-01 LPD

OFFICE OF THE SCIENTIFIC DIRECTOR, NIAID
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Z01 AI

Project Number

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Summary of Program
Laboratory and Clinical Research, NIAID
October 1, 1983 - September 30, 1984

In the pages that follow, the individual research summaries describe the research of the 13 laboratories which constitute the Intramural Research Program (IRP) of the National Institute of Allergy and Infectious Diseases. Ten of the laboratories are located at the NIH Campus in Bethesda while the remaining three laboratories are located at the Rocky Mountain Laboratories facility in Hamilton, Montana. The Office of the Scientific Director (OSD) is responsible for the administrative management of the Intramural Research Program, both in Montana and Bethesda.

During the past year, administrative changes were made to provide increased support for and coordination of the various research activities of the IRP by moving some support and coordination functions into the Office of the Scientific Director. Laboratory components which will provide services in peptide and oligonucleotide synthesis, fluorescent antibody cell sorting and clinical immunology of AIDS were transferred. In addition, a unit to coordinate all intramural activities in the area of AIDS research was established within this Office. It is planned that all these activities will be formally established as the Science and Technology Section of the OSD. This Section, along with the Animal Care Section and the Administrative Section will constitute the organizational structure of the OSD.

In response to requests from scientific and administrative staff, the Office of the Scientific Director has made an effort to supply microcomputers for support of research and administrative activities. It is our goal to eventually be able to provide microcomputers for the use of all senior scientific staff. We have done so for approximately one-fourth of the tenured scientists and will hope to respond to all requests within the next two years. Personnel records have been moved to microcomputers and efforts are underway to speed up our financial management and budget analyses through use of microcomputers.

A great deal of progress has been made by the IRP during the past year in AIDS research, the number one health priority of the Public Health Service. Approximately 20 projects have been initiated in the IRP specifically to study various aspects of this disease; an additional six projects are concerned partially with AIDS. In addition, work is nearing completion on a primate facility at RML to house chimpanzees for AIDS research. Dr. Aftab Ahmed Ansari, formerly Scientific Director of the Naval Medical Research Unit #3 in Cairo has been recruited to develop a program in molecular immunology at RML with emphasis on AIDS research and primate studies. A major accomplishment within the immunology unit of the OSD has been the ability to grow in large quantity the viral agent presumed to cause AIDS. A P3 level laboratory is being set up in building 550 at Ft. Detrick for large scale growth of this virus.

Outstanding research accomplishments were made throughout the laboratories of the IRP during the past year. Further application of

the vaccinia approach to vaccine development for a number of viral diseases has been underway. The DNA which codes for the protective antigen of hepatitis B has been inserted into vaccinia and this recombinant appears promising as a vaccine in animal studies. This approach is also under investigation for the development of an AIDS virus vaccine. Attempts are being made to clone the DNA which codes an envelope protein which is thought to have potential for protection. When successful, it will be inserted into vaccinia virus and tested in animal models. Major advances have been made toward the understanding of the biochemical basis of antigen-specific T cell activation and the role played by immune response genes in the regulation of this event. A new rotavirus vaccine has also been developed which appears to have great potential. Discussions are underway with commercial firms for further development, packaging and testing of this vaccine. The DNA coding for a potential protective antigen of the malaria parasite has also been cloned; a major step in the development of an effective and safe malaria vaccine. A new research program to develop an improved pertussis vaccine using recombinant DNA technology has been established at RML. Dr. Jerry Keith, who went to RML from the LID, has lead this effort with considerable success.

Equal Employment Opportunity and Affirmative Action Programs have been given considerable attention by OSD during the past year. Conscious efforts to recruit minorities have resulted in the addition of a number of minority staff members and fellows over the past year. The NIAID-IRP Introduction to Biomedical Research Program continues to be a model program within NIH. Forty nine students participated in the program this year and 24 spent the summer working in research laboratories of the NIAID and other NIH Institutes.

Safety in the laboratories has been stressed during the past year. A new Safety Committee was established under the chairmanship of Dr. Philip Baker of the LMI. The Committee is serving in a very conscientious manner and will be working to initiate a new program to systematically review and approve the use of new infectious agents within intramural laboratories. There has been some concern during the past year with problems related to radiation safety. Several intramural scientists temporarily lost their approval to use radioactive compounds and an aggressive attack on the problem has made considerable progress in improvement of the situation.

Considerable thought and planning have been devoted to the initiation of an expanded vaccine development program for the coming years. This will take the form of increased collaboration and cooperation with private industry but may also involve the development of a major intramural laboratory facility devoted to the development, production and testing of new and improved vaccines.

Planning has begun for the next step of the NIH "round robin" renovation project. Plans for the demolition and renovation of Building 4 are being developed by the NIH Engineering Design Branch, contract architects and NIAID intramural laboratory chiefs affected by the move. It is anticipated that the move will occur in the summer of 1987 at which time Building 5 will be vacated for renovation.

The NIAID Board of Scientific Counselors reviewed the Laboratories of Microbial Immunity, Immunogenetics and Infectious Diseases during the past year. The high quality of scientific research in the IRP continues to be recognized in these evaluations. The LIG and LID were particularly complemented for excellent research programs and accomplishments.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00383-02 OSD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Acquisition of Specimens from Cases of Acquired Immune Deficiency Syndrome

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Lois A. Salzman, Ph.D., OSD/NIAID

COOPERATING UNITS (if any)

Dr. Louis Baker, New York Blood Center; Dr. Jonathan Gold, Memorial Sloan-Kettering Cancer Center

LAB/BRANCH

Office of the Scientific Director, NIAID

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

0.5

PROFESSIONAL

0.5

OTHER

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The recent identification of the retrovirus HTLV III/LAV as the probable cause of AIDS has shifted our focus from the causative agent to its control, treatment and prevention. It is now important to define the early and late clinical, virologic, serologic and immunologic events associated with HTLV III/LAV infection. Questions to be answered include a knowledge of long term manifestations of infection with HTLV III/LAV prior to development of diagnosed AIDS and in patients who have antibody to the virus but have not developed defined AIDS or pre-AIDS. The intramural research contract with NYBC and Memorial Sloan-Kettering was designed to collect specimens in a prospective fashion from 325 homosexual males. Three populations were recruited: (1) patients with lymphadenopathy (100), (2) normal plasma donors (175) and (3) normal homosexual males from a geographically distinct area in New York State (50). Specimens of peripheral blood leukocytes, plasma, serum, urine, saliva and stool are being collected at intervals along with epidemiological information. During the first year of the contract, five participants in group 1 or 2 have been diagnosed as having AIDS. We are currently awaiting the results of ELISA tests for antibody to HTLV III/LAV in the serum of participants from group 1, 2 and 3. Using these specimens collected over several years, it may be possible to correlate the presence of HTLVIII/LAV antibody to clinical disease, to locate and isolate the virus from several sources and to gain information about changes in immune function in participants with antibody to HTLV III/LAV who do or do not develop AIDS.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00420-01 OSD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immune Complex Interaction in Immunoregulation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Kenneth W. Sell, M.D., Ph.D., NIAID/OSD
Lightfoote, Marilyn M., Ph.D., NIAID/OSD
Folks, T. M., Ph.D., NIAID/OSD

COOPERATING UNITS (if any)

Dr. Robert Redfield, Walter Reed Army Hospital; Dr. Jonathan Gold, Memorial Sloan-Kettering; Dr. Thomas Lawley, NCI

LAB/BRANCH

Office of the Scientific Director

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy & Infectious Diseases, NIH, Bethesda, Md. 20205

TOTAL MAN-YEARS:

1.50

PROFESSIONAL:

1.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Immune complexes from patients are being detected and studied by two techniques. The classical Raji assay using I¹²⁵ anti Ig and a modification of this technique using FITC-anti Ig. The latter method detects immune complexes by fluorescence using flow cytometry. Components within immune complexes have been separated and analyzed by gel electrophoresis. In addition, immune complex isolation and western blotting techniques are being applied to detect viral antigens within the complexes of patients with AIDS disease as well as other diseases.

The mechanisms by which immune complexes regulate immune responses are also being examined along with interactions at the cell surface between complement components and membrane components. Antigen-excess complexes and antibody-excess complexes are being constructed and used to study their regulatory influences on immune functions. The involvement of idiotype in the generation of immune complexes will also be investigated.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 A1 00417-01 OSD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Systematics and Vector Relationships of Ticks (Ixodoidea)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

James E. Keirans, Research Entomologist (Medical)

NIAID/OSD, Smithsonian Institution

COOPERATING UNITS (if any) Dr. H. Hoogstraal, NAMRU-3, Cairo; Drs. D. Sonenshine & P. Homsher, Old Dominion Univ.; Dr. J.B. Walker, Vet. Res. Service, Onderstepoort; Dr. R.G. Pegram, Tick Disease Unit, Lusaka, Zambia; Dr. C.E. Yunker, Washington State University, Pullman

LAB/BRANCH

Office of the Scientific Director

SECTION

Entomology Dept., Museum Support Center, Smithsonian Inst., Washington, D.C.

INSTITUTE AND LOCATION

National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

PROFESSIONAL

OTHER

2.0

1.0

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

The activities of this project currently comprise three main functions: (1) Identification of ticks received from various individuals and government agencies throughout the world. Only one other institution is capable of performing this service; (2) Systematic treatment, including taxonomy and classification, of the Ixodoidea, worldwide; (3) Entry, retrieval and use of tick data in the Smithsonian data processing system through installation of a terminal in the new tick project facility.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00422-01 OSD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Epidemiologic and Immunologic Studies on AIDS in Zaire

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Henry R. Francis, M.D., Expert, OSD/NIAID

COOPERATING UNITS (if any)

Dr. Jon Mann, Centers for Disease Control; American Embassy, Kinshasa

LAB/BRANCH

Office of the Scientific Director

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Acquired immune deficiency syndrome (AIDS) occurs at a very high rate in Zaire and attacks females at approximately the same rate as males. It is clear that the epidemiology of the disease in Africa differs from that seen in the United States and studies of the disease in Zaire could lend valuable information in understanding and combatting the disease in this country. For this reason, a Collaborative AIDS Project in Zaire (CAPZ) has been established by the Centers for Disease Control and the NIAID to study epidemiologic and immunologic aspects of this disease in Zaire. Dr. Jonathan Mann, CDC, arrived in Zaire in the Spring of 1984 and Dr. Henry Francis, NIAID, arrived in mid-September, 1984. The study is being set up, laboratory facilities prepared and equipment being shipped. Dr. Francis will be responsible for the laboratory aspects of the project and for some epidemiologic studies of special interest to the NIAID and Dr. Mann will be responsible for most epidemiologic study design and implementation. It is planned that a clinician will be added to the project in the near future. The project is estimated to take two years to complete.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00172-06 OSD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Structure and Synthesis of Peptides

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Walter Lee Maloy, Expert, OSD, NIAID

John E. Coligan, Research Chemist, LIG, NIAID

COOPERATING UNITS (if any)

Linda Smith, Univ. of Florida; Malcolm Martin, LMM, NIAID; Tom Kindt, LIG, NIAID; Lou Miller, LPD, NIAID; Ron Schwartz, LI, NIAID; Brian Murphy, LID, NIAID

LAB/BRANCH

SECTION

Office of the Scientific Director

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

PROFESSIONAL

OTHER

3.0

1.0

2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

Through protein and DNA sequence analysis, much information on the primary structure of proteins has accumulated over the past several years. We have used this information to prepare antibodies reactive with defined portions of proteins. This is done by synthesizing 10-30 residue peptides that correspond to sections of the sequence of the protein. After coupling to a protein carrier, antisera to the peptides are prepared. In the case of transplantation and other lymphocyte antigens, these antibodies are used to define sites of cellular recognition and serological specificities. In the case of viral antigens, the antisera have been used as specific probes to detect the expression of endogenous retroviral DNA found in human cells and will be used to attempt to enhance the immune response to a new flu vaccine. In some cases, the rabbit anti-peptide sera fail to react with the native protein and, therefore, anti-peptide hybridomas are being made and the hybrid clones screened for reactivity with native protein. Anti-peptide sera against the complementarity determining regions (CDR) of an immunoglobulin have been prepared and will be tested for their ability to kill the parent myeloma. Finally, peptides have been synthesized to be used to define the epitopes recognized by monoclonal antibodies to the p. falciparum sporozoite and the cytochrome C molecule.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 A1 00228-03-OSD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Flow Cytometric Analysis of Cell Membrane Antigens & Receptors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

M.J. Waxdal, Senior Investigator, OSD/NIAID

COOPERATING UNITS (if any)

Claudine Kiedo and Michel Monsigny, Centre National de Recherche Scientifique, Orleans, France; Nathan Sharon, Weitzman Institute, Rehovoth, Israel

LAB/BRANCH

Office of the Scientific Director

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:

1.9

PROFESSIONAL:

0.4

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Lymphoid cells possess cell surface glycoconjugates which can be used as differentiation markers and also serve as receptors for mitogenic lectins. It has recently been shown that lymphoid cells also bear surface receptors for carbohydrates (endogenous lectins) similar to those found in different cell types in various species. These endogenous lectins are excellent candidates for active participation in cell-cell recognition, homing, intracellular signaling and may also serve as a new set of differentiation markers.

Our prior studies have shown that both human and murine lymphoid cells bear specific endogenous lectins. That these markers may be used to develop physical methods of cell separation and that these endogenous lectins do appear to be involved in lymphocyte differentiation.

This year, almost the entire effort has been directed to establishing an Institute flow cytometry facility and in organizing and conducting two international symposia in this field.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00421-01 OSD

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Analysis of Specimens Collected from Populations at Risk of AIDS

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

M.J. Waxdal, Senior Investigator, OSD/NIAID

Lois Salzman, Contract Officer, OSD/NIAID

Albert Saah, Medical Epidemiologist, ESB/MIDP/NIAID

Richard Kaslow, Chief, EBS/MIDP/NIAID

COOPERATING UNITS (if any) Louis N. Baker, N.Y Blood Center; Jonathan Gold, Memorial Sloan-Kettering; Roger Detels, UCLA; David Ostrow, Howard Brown Memorial Clinic, Chicago; Frank Polk, Johns Hopkins; Charles Renaldo, U. Pittsburgh; Warren Winkelstein, UC, Berkeley; Jim Leaf, BRI, Rockville, Md.

LAB/BRANCH

Office of the Scientific Director

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD

TOTAL MAN-YEARS

0.6

PROFESSIONAL

0.6

OTHER

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

To establish, implement and maintain the Repository Data Center for the joint AIDS contracts effort between the Intramural Research Program (OSD/IRP) and the Microbiology and Infectious Disease Program (MIDP). This effort is a study of the epidemiology, pathogenesis, natural history, susceptibility, and causative agents of Acquired Immune Deficiency Syndrome (AIDS). The data base will contain information on approximately two million medical specimens from six contractors.

Specimens from selected patients will be sent to qualified investigators for special testing and biological research. The data on these removals from the repository, on the tests and research to be performed, and the results also will be entered and maintained in the database.

To coordinate and evaluate flow cytometry studies of patient PBL by the AIDS contractors.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00385-02 OSD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunologic Evaluation of Chimps Exposed to Materials from AIDS Patients

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Thomas Folks, Ph.D., NIAID/OSD

COOPERATING UNITS (if any)

Dr. Robert Purcell, NIAID/LID; Dr. Kenneth Sell, NIAID/OSD

LAB/BRANCH

Office of the Scientific Director

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy & Infectious Diseases, NIH, Bethesda, Md. 20205

TOTAL MAN-YEARS:

PROFESSIONAL

OTHER

1.825

0.075

1.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A colony of chimpanzees currently housed at Maloy Labs are being evaluated for a number of viral studies. Many of the chimpanzees have been inoculated with blood from AIDS patients and followed by weekly helper/suppressor ratios for over 15 months. No changes have occurred. In addition, lymphocytes from these and other normal chimps have been co-cultured with AIDS virus to determine the host range of susceptible chimps to the virus. Greater than 80% of the chimps show slowed growth following in vitro virus infection of T blast cells. Only one chimp's lymphocytes has actually produced detectable reverse transcriptase activity which was found on day 24 post infection. Further studies are being conducted in order to determine dose and kinetics of in vitro and in vivo AIDS virus infectivity and production.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00384-02 OSD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Evaluation of Immunologic Abnormalities in AIDS Patients

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Thomas Folks, Ph.D., NIAID/OSD

COOPERATING UNITS (if any)

Dr. Thomas Chused, LMI/NIAID; Dr. Robert Redfield, Walter Reed Army Hospital

LAB/BRANCH

Office of the Scientific Director

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy & Infectious Diseases, NIH, Bethesda, Md. 20205

TOTAL MAN-YEARS

0.9

PROFESSIONAL

0.15

OTHER

0.75

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Lymphocytes from patients suffering from AIDS or AIDS Related Complex (ARC) have been evaluated for surface phenotype changes during the course of their disease. In all cases a subpopulation of helper T Lymphocyte (the inducer of cytotoxic/suppressor cells, Leu 8) has been shown to selectively be depleted from peripheral blood of these patients. Further studies are underway to better characterize the nature of this subpopulation loss. Heterologous antisera as well as monoclonal antibodies to the AIDS virus are being prepared and will be used as a surface probe to define by two and three color FACS analysis the cells which are infected. Two color quantitative isolation of the antibody positive cells can then be analyzed with DNA probes made from the AIDS virus to determine if these cells bear proviral DNA.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00419-01 OSD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Immunobiology of AIDS Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

T. M. Folks, Ph.D., Expert, OSD, NIAID

COOPERATING UNITS (if any)

Dr. Malcolm Martin, NIAID/LMM; Dr. John Sogn, NIAID/LIG; Dr. Robert Redfield
Walter Reed Army Hospital; Dr. M. Lightfoote, NIAID/OSD; Dr. Kenneth Sell,
NIAID/OSD

LAB/BRANCH

Office of the Scientific Director

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy & Infectious Diseases, NIH, Bethesda, Md. 20205

TOTAL MAN-YEARS

3.375

PROFESSIONAL

0.375

OTHER

3.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

Characterization of AIDS virus isolates and their growth in permissive cells is currently underway. New cell lines are being evaluated for their capability in becoming infected with and producing human retroviruses suspected as the cause of AIDS. Analysis of the viruses will be accomplished by western blot or ELISA with AIDS patients sera and heterologous or monoclonal antibody currently being produced. Other investigations underway are those of understanding the cellular biology of the growth of the viruses in both normal human T cells and tumor cell lines. These studies utilize reverse transcriptase detection FACS analysis, and cellular morphology as methods of evaluation. Development of CPE and neutralization assays are also being investigated in order to study the cellular biology of these viruses.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00071-13 OSD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Studies on Pertussigen

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

J.J. Munoz, Research Microbiologist, OSD/NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Office of the Scientific Director

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

1.0

PROFESSIONAL

1.0

OTHER

none

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

Pertussigen (pertussis toxin) is the active principle in pertussis vaccine. It has many biological activities of interest among which its immunopotentiating activities are outstanding. In nanogram amounts it enhances production of the IgE antibodies, promotes the induction of experimental allergic encephalomyelitis, enhances delayed type of hypersensitivity, and increases inflammation caused by immunological reactions. Pertussigen is made up of 5 peptides which are distinct as shown by their peptide composition following chymotrypsin digestion. In SDS-PAGE the 5 peptides have molecular weights of 28, 23, 22, 11 and 9 K daltons, but upon reduction with 2ME four of these peptides show an apparent increase in molecular weight. Proteinase K or subtilisin digestion of pertussigen does not change its biological activities, but it does digest the 28 K and the 9 K dalton subunit. The significance of each subunit peptide is presently under study, and monoclonal antibodies have been obtained for 3 peptides. With these specific antibodies, the role of each subunit in the various biological activities will be investigated.

| | | |
|---|----------------------------|---------------------------------------|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00182-06 OSD |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biochemical & Genetic Mechanisms of Obligate Intracellular Parasitism | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) J.C. Williams, Ph.D., Sr. Scientist, NIAID/OSD, Group Leader, Rickettsial Diseases Laboratory, USAMRIID E.H. Stephenson, DVM, Ph.D., COL, VC, Chief, Aerobiology Division, USAMRIID M.H. Vodkin, Ph.D., USAMRIID C.E. Snyder, Jr., Ph.D., CPT, MSC, USAMRIID | | |
| COOPERATING UNITS (if any) K. -I. Amano, Hirosaki University School of Medicine, Hirosaki, Aomori, Japan | | |
| LAB/BRANCH Office of the Scientific Director SECTION | | |
| INSTITUTE AND LOCATION National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20205 | | |
| TOTAL MAN-YEARS 1.6 | PROFESSIONAL 1.1 | OTHER .5 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Lipopolysaccarides (LPS), isolated from phase I and phase II <i>Coxiella burnetii</i>, were analyzed. Purification of LPS by ultracentrifugation gave similar yields for both LPSI and LPSII. Purified LPSI and LPSII contained roughly 0.8 to 0.6% protein. The fatty acid constituents of the LPSs were different in composition and content, with branched chain fatty acids representing about 15% of the total. Beta-hydroxymyristic acid was not detected in either LPSI or LPSII. A thiobarbituric acid-periodate positive compound was evident in the LPSs, however, this compound was not identified as 3-deoxy-D-mannooctulosonic acid by gas and paper chromatography. LPSII contained D-mannose, D-glucose, D-glyceromannoheptose, glucosamine, ethanolamine, 3-deoxy-mannooctulosonic acid-like material, phosphate, and fatty acids. LPSI contained a unique disaccharide galactosaminuronyl-glucosamine and nine unidentified components in addition to the same components of LPSII. Analysis of LPSs by SDS-PAGE followed by silver staining indicated that LPSII was composed of only one band, while LPSI consisted of six or more bands with irregular spacing. </p> <p> Chromosomal DNA has been extracted from Nine Mile phase I <i>Coxiella burnetii</i>. Chromosomal DNA has been cloned in a g-based vector system of <i>E. coli</i>. A library of 150 plaques, representing about 1750 kilobases (kb), has resulted. The evidence suggests that rickettsial DNA is being expressed in at least some of these clones. A 36 kb plasmid Q phase I has been previously described in <i>C. burnetii</i>. It has been successfully extracted from several different strains (phase I and phase II). Restriction patterns so far appear almost identical, though there may be some subtle differences. This plasmid (from the Henzerling strain) has been cloned in the <i>E. coli</i> plasmid vector pBR322. </p> | | |

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00183-06 OSD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders)

Immunologic Properties of *Coxiella burnetii* (Q Fever) Vaccine

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

J.C. Williams, Ph.D., Sr. Scientist, NIAID/OSD, Group Leader Rickettsial Diseases Laboratory, USAMRIID
 E.H. Stephenson, DVM, Ph.D., COL, VC, Chief, Aerobiology Division USAMRIID
 C.E. Snyder, Jr., Ph.D., CPT, MSC, USAMRIID
 G.S. Horwith, M.D., MAJ, MC, USAMRIID
 G.H. Scott, Ph.D., USAMRIID
 V.S. Sanchez-Carlo, Ph.D., NRC Post-Doctoral, USAMRIID
 D.M. Waag, M.S., Ph.D. Candidate, NIAID/OSD

COOPERATING UNITS (if any)

K. -I. Amano, Hirosaki University School of Medicine, Hirosaki, Aomori, Japan

LAB/BRANCH

Office of the Scientific Director

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

PROFESSIONAL

OTHER

4.3

3.3

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Subfractions of *Coxiella burnetii*, the etiologic agent of Q fever, which confer immunological efficacy as measured by humoral and cellular mechanisms have been investigated. Attempts to demonstrate the chemical nature of the reactogenic components of *C. burnetii* suggest that carbohydrate and lipid are involved in the Q-complex which induces mitogenic hyporesponsiveness and negative modulation of cellular immunologic functions. Using inbred strains of mice a model for the study of resistance to infection and reactogenic activities of candidate vaccines has been established. Activation of murine peritoneal macrophages to perform non-specific tumoricidal cell cytotoxicity was assayed using phase I *C. burnetii* whole cell (WC) and chloroform-methanol residue (CMR) vaccines. Macrophages from mice resistant, intermediately sensitive and sensitive to *C. burnetii* infection were activated by WC vaccine, whereas CMR vaccine was less effective as a non-specific activator of macrophages. Murine monoclonal antibodies against phase I *C. burnetii* lipopolysaccharide (LPS) were produced. Iodination of phase I and phase II WCs revealed a major outer membrane protein of about 29.5K. This surface exposed protein was extracted in an aqueous buffered detergent system, purified to homogeneity by column chromatography and preparative sodium dodecyl sulfate polyacrylamide gel electrophoresis and determined to be an immunodominant antigen using immunoblotting, enzyme-linked immunosorbent assay and radioimmune precipitation. A phase 2 clinical trial using phase I WC vaccine was initiated and should be completed within a few months so that a phase 3 clinical trial can be initiated and should be completed within a few months so that a phase 3 clinical trial can be initiated in early 1985. Currently, the detoxified CMR derived from phase I WC is being produced so that phase I testing of this candidate Q fever vaccine can be initiated.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00191-06 OSD

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunoregulation of T Lymphocytes by Immune Complexes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Kenneth W. Sell, Scientific Director, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

OSD

SECTION

Science and Technology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

.4

PROFESSIONAL:

.2

OTHER:

.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Murine T cell hybridomas for ovalbumin (OVA) and insulin have been produced and tested for ability to regulate the responses of specific B cells. Several categories of T cell hybridomas have been described. Some kinds of T cell hybridomas secrete IL-2 specifically when stimulated with the correct antigen, insulin or OVA, and in addition, help OVA-specific histocompatible B cells or TNP-specific histocompatible B cells make antibodies while others do not help specific B cells make antibodies. Other T cell hybridomas are self reactive but not antigen specific and these augment suboptimal numbers of antigen primed T cells in B cell secretion assays. For the insulin system, B cell hybridomas antibodies have been used to prepare anti-idiotypic reagents that do not affect IL-2 secretion by anti-idiotypes clearly does produce regulatory effects in an insulin immune response when examined in in vivo or in vitro assays. Future work will involve further experiments to define cellular control of the network activated by this anti-idiotypic. In other recently initiated experiments, human-mouse T cell hybrids are being used to study new human surface molecules at both the protein and gene levels.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00378-01 OSD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Attempted Isolation of AIDS Agent from High Risk Specimens

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Robert M. L. Buller, OSD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Office of the Scientific Director

SECTION

Office of the Scientific Director

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD

TOTAL MAN-YEARS

PROFESSIONAL

OTHER

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

THIS PROJECT HAS BEEN TERMINATED.

LABORATORY OF BIOLOGY OF VIRUSES

1984 Annual Report

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Z01 AI
Project Number

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LABORATORY OF BIOLOGY OF VIRUSES

National Institute of Allergy and Infectious Diseases

SUMMARY - October 1, 1983 - September 30, 1984

The past several years have seen a significant change in the focus of work within the Laboratory of Biology of Viruses. Recombinant DNA technology and DNA sequencing techniques have permitted the examination of conceptionally new problems. For most of the small viruses, precise knowledge of virion structure has been available but recombinant methodology has allowed detailed analysis of regions of the larger viruses to be defined. Using data obtained by DNA sequencing, information on viral specified RNA can now be compared with the DNA template from which it was copied. This has provided an understanding of how simple genomes can generate multiple viral proteins by using alternate splice sites to form overlapping but unique messenger RNA molecules. The precise localization of the start sites for transcription of specific genes has permitted the identification of regions that lie upstream from the start sites which regulate the rate of transcription. The localization of these control regions has allowed investigators to enhance or suppress expression of specific genes by introducing site specific mutations into these control regions.

A major goal of modern biology has been to define the relationship between the information contained within the DNA and its functional expression by a virus or a cell. Projects described in this year's annual report demonstrate that we are achieving that goal.

Laboratory of Biology of Viruses
National Institute of Allergy and Infectious Diseases

SUMMARY - October 1, 1983 - September 30, 1984

HONORS AND AWARDS

Dr. Norman P. Salzman continued to serve on the Editorial Board of the Journal of Virology, and as Professorial Lecturer, Georgetown University School of Medicine.

Dr. James Rose was appointed as an Associate Editor of the journal, Virology (January, 1984).

Some of the highlights of this year's research efforts are detailed below.

PAPPOVAVIRUSES

A. Mutants that Effect Transcription of the Early Genes of SV40 Define A New Control Region

A set of nine mutants containing point mutations, and small deletions or insertions, were constructed in the early promoter region of SV40 to determine the role of the DNA sequences between the TATA box and the six upstream GC rich clusters in early transcription. The mutant templates were tested for in vitro transcription in Hela cell extracts and in vivo in CV1 and COS cells using the chloramphenicol acetyl transferase gene (CAT) assay. Both in vitro and in vivo results show that the narrow region between nucleotide position (np) 38-41 is an important domain of the early promoter. Deletion and insertion mutations most strongly affect the level of transcription. While a four base pair deletion in the promoter region enhances the level of transcription four to six-fold in vitro, it causes a four fold suppression of CAT gene expression in an in vivo assay. These opposite effects may result from changes in spacing under in vitro and in vivo conditions between two domains where transcription factors make simultaneous contact. Of the three T-antigen binding sites (I, II, III) sites I and II have already been shown to be involved in the autoregulation of the early transcription. Mutational analyses has demonstrated the role of site III, which partially overlaps with np 38-41, in the autoregulation of the SV40 early promoter.

B. There are Regulatory Domains that Functoin Separately to Control the Level of the Late Genes of SV40

An 11-base DNA sequence, 5'-G-G-T-A-C-C-T-A-A-C-C-3' (simian virus 40 [SV40]map position 294 to 304), which is important in the control of SV40 late RNA expression in vitro and in vivo has been previously identified. We have since defined another domain of the SV40 late promoter. A series of mutants with deletions extending from SV40 map position 0 to 300 was prepared by nuclease Bal31 treatment. The cloned templates were then analyzed for efficiency and accuracy of late SV40 RNA expression in the Manley in vitro transcription system. These studies have shown that, in addition to the promoter domain near map position 300, there are essential DNA sequences between nucleotide positions 74 and 95 that are required for efficient expression of late SV40 RNA. Included in this SV40 DNA sequence were two of the six GGGCGG SV40 repeat sequences and an 11-nucleotide segment which showed strong homology with the upstream sequences required for the efficient in vitro and in vivo expression of the histone H2A gene. This upstream promoter sequence supported transcription with the same efficiency even when it was moved 72 nucleotides closer to the major late cap site. In vitro promoter competition analysis demonstrated that the upstream promoter sequence, independent of the 294 to 304 promoter element, is capable of binding polymerase-transcription factors required for SV40 late gene transcription. DNA sequences which control the specificity of RNA initiation at nucleotide 325 lie downstream of map position 294.

C. The Sequences of JC Virus that Confer Host Range Specificity Have Been Identified.

JCV, a human polyoma virus, causes the fatal demyelinating disease progressive multifocal leukoencephalopathy. The virus has an extremely restricted host range and grows well only in human fetal glial cells. The two 98 bp tandem repeats located upstream of the origin of replication in the JC genome have been cloned into the pCAT3M plasmid, which has the bacterial chloramphenicol acetyl transferase gene. The JC tandem repeats, which share significant homology with an 82 nucleotide rat brain specific RNA sequence, were shown to contain stronger enhancer-like activity in fetal glial cells than in other cell types. The narrow host range of JCV is at least partly the result of restricted enhancer activity.

PARVOVIRUSES

A. Coding Sequences for the AAV Proteins Have Been Mapped

It was previously demonstrated that adeno-associated virus (AAV) contains three structural protein species: A, B, and C (90, 72 and 60 kilodaltons [kd]), in the case of AAV2). In addition, four distinct subspecies of C and two of A. The three primary capsid proteins also have been shown to contain overlapping amino acid sequences (R. McPherson and J. Rose, J. Virol. 46:523-529, 1983). Definitive evidence has now been obtained that these proteins are encoded in the right half of the AAV2 genome, and one or both of the smallest AAV RNA species [2.3 or 2.6 kilobase (kb) RNA] account for their synthesis. Protein A (90 kd) apparently initiates from a site within the intervening sequence (IS), which is intact in the larger (unspliced) 2.6 kb mRNA, and may read through one or more termination codons, including a strong stop signal (UAA) that lies thirty-one bases downstream from the end of the IS. Proteins B (72kd) and C (60kd) are not derived from protein A but apparently originate from independent, in-frame initiations that lie downstream from the splice junction. It thus seems likely that production of the three AAV capsid proteins involves at least two mRNA species. The B and C proteins presumably arise from the spliced 2.3kb RNA, whereas protein A should be generated by the 2.6kb RNA or a hitherto unidentified spliced RNA species.

To confirm the independent origins of B and C, their positions were directly mapped with respect to the reported nucleotide sequence. Partial sequence analysis of selected tryptic peptides reveals that the origin of C corresponds to the AUG codon at map unit position 60, and that protein B is initiated in-frame at a triplet 195 nucleotides upstream from the C origin. Sequence data also confirms the extensive amino acid overlap among the A, B and C proteins. Taken together, these findings demonstrate that the three capsid components arise from separate, in-frame initiation codons. Currently, we are trying to locate the A origin site as well as the specific mRNA that accounts for this protein.

B. Synthesis of AAV DNA Has Been Studied in Vitro

The overall scheme of AAV DNA synthesis *in vivo* was first described in this laboratory (Straus et al., Proc. Natl. Acad. Sci. 73, 742-746, 1976).

Briefly, following coinfection of KB cells with AAV and a helper Ad, AAV DNA synthesis is initiated on single-stranded genomic templates by a self-priming mechanism. Subsequent elongation yields a unit length hairpin intermediate. A second round of self-primed synthesis displaces the 5'-ended arm of the hairpin and leads to either (i) displacement of a complete plus or minus progeny strand (by virtue of a processing/synthesis step at the closed end of the hairpin) or (ii) concatemeric molecules if closed end processing/synthesis does not occur. These latter molecules can be eventually processed to unit length templates which, in turn, may also yield progeny strands by new self-primed rounds of displacement synthesis. It has been suggested that a similar synthetic mechanism may be involved in the replication of cellular DNA. At present, the specific enzymatic and regulatory factors (both cellular and viral) that participate in AAV DNA synthesis are not clearly defined. To help identify and characterize these elements, we have utilized in vitro DNA synthesizing systems that generate AAV DNA replicating forms which correspond to those found in vivo. One of these systems consists of adenovirus-infected cytosol, uninfected nuclear extract and a DNA-protein template released from purified AAV virions. Analyses of the in vitro synthesized products reveals the presence of progeny DNA strands, duplex unit-sized hairpin molecules (replicative form [RF]DNA) and concatemeric structures. An initial replicating intermediate which precedes self-primed synthesis of duplex RF molecules has also been identified. This mode of replication differs from that catalyzed by E. coli DNA polymerase I and provides further insight into the self-priming mechanism for AAV DNA replication.

C. The Role of the Adenovirus VA Genes in Protein Syntheses

It was previously shown that the VAI RNA gene was required for replication of defective parvoviruses (AAV; Janik et al., Proc. Natl. Acad. Sci. USA 78:1925-1929, 1981). We have now shown that the VA gene products (relatively short RNA molecules, approximately 160 nucleotides in length) exert their effect at the level of translation and apparently do so in conjunction with a second early Ad gene product, the DNA-binding protein (DBP). In addition, we also have observed that DBP alone can modulate translational expression of specific Ad and AAV mRNAs. In other work complementary activity of SV40 T antigen for an Ad DBP mutant (DNA minus) appears to occur, although at low levels.

Current studies are directed at determining the mechanism by which Ad VA RNA(s) enhances translational expression of AAV mRNA species. We have now developed sensitive probes to specifically detect the individual VA RNA species (VAI or VAII). Their relative efficiencies in enhancing AAV translation are being analyzed.

D. Characterization of Kilham Rat Virus (KRV) Transcription Products.

We have isolated from and partially characterized five functional KRV specific mRNAs from polysomes in infected cells. The longest KRV RNA is 4.7 kilobases (kb) and represents a transcript of 95 to 100% of the viral genome. The most abundant KRV-RNA is 3.0 kb representing about 50% of the viral genome and probably codes in the reticulocyte transcribing system for the most abundant viral protein (m.w. 68,000). We have mapped this 3.0 kb RNA on

the viral genome and have determined the origin of transcription. We have cloned some of the KRV genome and hope to finish the nucleotide sequence of the genome.

E. Shared Regions of the Genome Specify KRV Proteins.

Since the KRV genome contains approximately 5,000 nucleotides, the maximal protein coding capacity is for protein(s) with a total maximal molecular weight of 187,000 ($5,000/3 \times 110$). The KRV capsid proteins with molecular weights of A (89,000) and B (65,000) would require the coding capacity of almost the entire genome. Since nonstructural viral proteins are present in the infected cell, the coding capacity of the viral genome must involve the use of some type of overlapping gene sequences. We determined by peptide analysis that the capsid proteins are translated in the same reading frame from overlapping sections of the genome. We used the Cleveland procedure with digestion by *S. aureus* V8 protease and peptide fingerprinting. The nine peptides found in fingerprinting protein B are all identical to nine of the 12 peptides found in the protein A fingerprint. Thus, there is either more than one promoter in the DNA sequence translated or the larger protein is processed after transcription.

F. DNA Replication of KRV.

We have found that the 5' terminus of the replicative form of KRV is covalently linked to a protein. Terminal proteins have been found linked to the DNA of several viruses, including adenovirus. The terminal protein may play some role in DNA replication but its function is still not known. We have isolated the KRV terminal protein, found its molecular weight to be about 68,000 and are determining its peptide map to compare it with the viral capsid proteins.

ADENOVIRUSES

A. Enhanced in Vitro Expression of SV40 and Adenovirus Promoters

The whole cell (Manley) extract is able to carry out transcription in vitro, and initiation of transcription occurs at the same 5' start sites that are used in vivo. However, this system does not reflect the level of promoter utilization that is observed in vivo, and some promoters that function in the cell are not expressed in vitro.

Transcription of certain SV40 and adenovirus genes can be selectively enhanced under suitable in vitro assay conditions. The major SV40 late promoters and the adeno IVa2 promoter do not have TATA box sequences upstream from the start sites of transcription. Analyses of transcripts synthesized in vitro from the SV40 late promoters by "run-off" and S1 nuclease mapping methods show that when transcription takes place in the presence of 15 mM (NH₄)₂SO₄, there is a 3- to 5-fold stimulation of RNA synthesis initiating from SV40 early promoters. RNA initiating from map position 325 is stimulated 40-fold under these conditions. When the (NH₄)₂SO₄ concentration is increased to 50 mM (NH₄)₂SO₄, there is a 90% suppression of transcription. Ammonium sulfate also enhances transcription from the adeno IVa2 promoter, which is not expressed in extracts that lack (NH₄)₂SO₄.

B. Control Signals that Regulate Expression of the IVa2 Gene In Vitro

The adeno IVa2 gene, that is expressed at an intermediate time in the viral infectious cycle, is separated from the adenovirus major late promoter (MLP) 5' start site by 210 base pairs and is transcribed from the opposite strand. In contrast to the MLP, the IVa2 gene does not contain a TATA box upstream from its 5' start sites. Using a series of deletion mutants, two upstream control regions that are rich in C-residues, one proximal to the cap site at np (nucleotide position) -39 to -48 and a distal domain between np -152 and -242 have been identified as essential for IVa2 transcription (IVa2 cap site is np +1). Transcription efficiency is decreased by 70 to 90% after the deletion of a proximal C-rich domain when either linear or supercoiled DNAs were used as template. However, distal sequences functioned as transcriptional control domains only with covalently closed DNA templates. When the plasmid pAd242 that contains the 5' start sites of adeno MLP and IVa2 is transcribed, there is essentially a complete suppression of transcription of the adeno IVa2 gene. The transcription efficiency of IVa2 is increased ten-fold after deletion of the MLP cap site. A model based on a shared entry site for RNA polymerase II and competition between major late and IVa2 promoters is proposed to explain the in vitro transcriptional results.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00290-03 LBV

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. The number 1 on one line between the borders.)
Deletion Mutants in Control Regions that Regulate SV40 TranscriptionPRINCIPAL INVESTIGATOR (List name, profession and personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)
PI: Norman P. Salzman, Ph.D.

| | | | |
|---------|----------------------|-----------------|------------|
| Others: | Helena Mishoe, Ph.D. | Staff Fellow | LBV, NIAID |
| | Asit Nandi, Ph.D. | Visiting Fellow | LBV, NIAID |
| | Charles Rodi, Ph.D. | Staff Fellow | LBV, NIAID |

COOPERATING UNITS (Name)

| | | |
|-------------------|--------------------|----------|
| John Brady, Ph.D. | Staff Fellow | LMV, NCI |
| Gokul Das, Ph.D. | Visiting Associate | LMD, NEI |

LAB BRANCH

Laboratory of Biology of Viruses

SECTION

Biochemical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

4.7

PROFESSIONAL

2.7

OTHER

2.0

CHECK APPROPRIATE BOXES

| | | |
|---|--|---|
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input checked="" type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors | | |
| <input type="checkbox"/> (a2) Interviews | | |

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

In most eucaryotic genes, a common set of nucleotides has been found before the start site of RNA transcription. These nucleotides, referred to as either a TATA or Goldberg-Hogness box, are important determinants of transcription in vivo and in vitro. The control region for the late SV40 transcripts have been examined by site specific mutagenesis and by generation of deletion mutations. Mutations that enhance or suppress the transcriptional activity of a single start site have been identified and differ from the consensus Goldberg-Hogness box. The presence in SV40 of a weaker late promoter as compared with the early promoter may be a mechanism to regulate genes in a temporal manner. By late genes having a lower affinity for RNA polymerase II, the synthesis of early transcripts that are required to initiate viral DNA replication are favored. An additional cluster of nucleotides that is located at base positions 75-96 also enhances the level of transcription and it maintains this enhancing effect when its position relative to the 5' start site is altered.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00291-02 LBV

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Interaction of RNA Polymerase II with Defined DNA Templates

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Norman P. Salzman, Ph.D.

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Biology of Viruses

SECTION

Biochemical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

PROFESSIONAL

OTHER

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

This project has been terminated.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00292-03

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders)

Factors Required for Specific Transcription

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Norman P. Salzman, Ph.D.

Chief

LBV, NIAID

Others: V. Natarajan, Ph.D.

Visiting Associate

LBV, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Biology of Viruses

SECTION

Biochemical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

2.6

PROFESSIONAL

1.2

OTHER:

1.4

CHECK APPROPRIATE BOX(ES)



(a) Human subjects



(b) Human tissues



(c) Neither



(a1) Minors



(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In vitro transcription systems are used to identify promoter signals and factors that control transcription in eucaryotic systems. These systems accurately initiate transcription from a wide variety of promoters. Preferential stimulation of certain promoters is achieved in the presence of low concentrations of ammonium sulfate. The addition of (NH₄)₂SO₄ allows detection of promoters which could not otherwise be detected and enhances in vitro expression of the late promoter 5' start site that is used as the principal in vivo start site. Under these modified conditions, expression of the adenovirus IVa2 gene is readily observed in vitro, while under standard conditions, it is not transcribed. This adeno gene, like the SV40 late genes, lacks a TATA box upstream from the 5' start site. By constructing a series of upstream deletion mutations, two upstream domains have been identified that controls this gene. The distal domain only functions when covalently closed DNA is used as a template.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00293-03 LBV

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Studies on the Replication of the Parvovirus KRV (Kilham rat virus)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Lois A. Salzman, Ph.D.

Research Chemist

LBV, NIAID

Others: Dale Brown, Ph.D.

Guest Worker

LBV, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Biology of Viruses

SECTION

Molecular Structure Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

3.3

PROFESSIONAL

2

OTHER

1.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type Do not exceed the space provided)

The parvovirus, KRV, is a member of an increasingly important group of small eukaryotic viruses. They contain a single stranded DNA molecule containing about 5000 nucleotides and two or three virion proteins. We have been studying the replication of this virus in the infected cell. We have isolated the viral specific RNAs, characterized them and used them in an erythrocyte translating system. We have studied the 5' DNA terminal protein characterizing it in an attempt to understand its function. We have also studied the relationship of the viral capsid proteins.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00294-03 LBV

PERIOD TO COVER

October 1, 1983 to September 30, 1984

* TITLE OF PROJECT (50 characters or less. The number is on one line between the borders.)

Structure and Function of Adenovirus DNA

PRINCIPAL INVESTIGATOR (List only professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: James A. Rose, M.D.

Section Head

LBV, NIAID

COOPERATING UNIVERSITY

Richard McPherson, M. D., Department of Pathology, Georgetown University Hospital, Washington, D. C.

LAB BRANCH

Laboratory of Biology of Viruses

SECTION

Molecular Structure Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

.7

PROFESSIONAL

.3

OTHER

.4

CHECK APPROPRIATE BOXES

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Among objectives of these studies has been the application of physical, biochemical and biological techniques to characterize the structure and functions of certain segments (i.e., the inverted terminal repeat) and genes of adenovirus (Ad) DNA. In earlier studies, we first identified and characterized the VA RNA gene/transcript and inverted terminal repeats in Ad DNA. We have continued to investigate the specific regulatory functions of several early Ad genes, e.g., the VA and DNA-binding protein genes. Recent results indicate that these latter genes are involved in the regulation of translation of certain viral mRNAs. Among methods used are gradient sedimentation, DNA cleavage with restriction endonucleases, gel electrophoresis, base sequence analysis and DNA transfection.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00295-03 LBV

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Helper Factors Required for Expression of the Adeno-Associated Virus Genome

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: James A. Rose, M.D.

Section Head

LBV, NIAID

COOPERATING UNITS // a- Richard McPherson, M.D., Dept. of Pathology, Georgetown University Hospital, Washington, D.C.; Leonard Rosenthal, Ph.D., Dept. of Microbiology, Georgetown University, Washington, D. C.; John Hay, Ph.D., Dept. of Microbiology, Uniformed Services University of the Health Sciences, Bethesda, MD

LAB/BRANCH

Laboratory of Biology of Viruses

SECTION

Molecular Structure Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

1.2

PROFESSIONAL

.3

OTHER

.9

CHECK APPROPRIATE BOXES:

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The main objectives of this project are (i) to define where and how each required helper virus factor regulates expression of defective human parvovirus (AAV) genomes and (ii) to relate these findings to their respective roles in the replication of the helper viruses (adenoviruses, herpesviruses) themselves as well as to potentials for selective interference with viral infection. We previously mapped the adenovirus genes required for AAV replication and continue to investigate their specific helper functions. In addition, we have recently demonstrated that human cytomegalovirus is a competent helper for AAV multiplication, and we are now attempting to map and characterize the required cytomegalovirus genes. Among methods used are specific immunofluorescence, cleavage of DNA with restriction endonucleases, DNA cloning, gel electrophoresis, blot-hybridization analyses and DNA transfection of cells.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00296-03 LBV

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization and Production of Parvovirus Proteins

PRINCIPAL INVESTIGATOR (List other professional persons below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: James Rose, M.D.

Section Head

LBV, NIAID

Others: Edwin Sebring, Ph.D.

Research Chemist

LBV, NIAID

Patricia Becerra, Ph.D.

Visiting Associate

LBV, NIAID

COOPERATING UNITS (if any)

- (1) Richard McPherson, M.D., Department of Pathology, Georgetown University Hospital, 3800 Reservoir Road, N.W., Washington, D. C. 20007;
(2) Carl W. Anderson, Ph.D., Brookhaven National Laboratory, Upton, New York 11973

LAB BRANCH

Laboratory of Biology of Viruses

SECTION

Molecular Structure Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

2.3

PROFESSIONAL

1.7

OTHER

.6

CHECK APPROPRIATE BOXES:

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The main objectives of these studies are (i) to identify and characterize all proteins that are specified by the defective human parvoviruses (AAV) and to determine similarities and differences with autonomous parvovirus proteins, (ii) to define the mechanism(s) by which the AAV proteins arise and (iii) to define specific functions of the AAV proteins. We have now identified several AAV non-structural proteins which were previously undetected. At least one of these proteins is necessary for viral DNA replication. Post-translational processing does not account for production of any AAV structural proteins, although they share large proportions of sequences-in-common. It is now clear, however, that these proteins originate from independent in-frame initiations. The mechanism that regulates translation of AAV proteins is of fundamental interest and is now being investigated. Among methods used are affinity chromatography, gel electrophoresis, in vitro translation of viral RNA, electrophoretic and HPLC analyses of V8 protease and tryptic peptides and aminoterminal sequencing of purified polypeptides.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00297-03 LBV

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanism and Regulation of Adeno-Associated Virus DNA Replication

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: James Rose, M.D. Section Head LBV, NIAID

| | | | |
|---------|----------------------|--------------------|------------|
| Others: | Er-Chung Wang, Ph.D. | Visiting Associate | LBV, NIAID |
| | Edwin Sebring, Ph.D. | Research Chemist | LBV, NIAID |
| | Seigo Ohi, Ph.D. | Visiting Associate | LBV, NIAID |

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Biology of Viruses

SECTION

Molecular Structure Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

3.6

PROFESSIONAL

2.8

OTHER

.8

CHECK APPROPRIATE BOX(ES)

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|---|---|--------------------------------------|
| <input type="checkbox"/> (a) Human subjects | <input checked="" type="checkbox"/> (b) Human tissues | <input type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors | | |
| <input type="checkbox"/> (a2) Interviews | | |

SUMMARY OF WORK (Use standard, unreduced type. Do not exceed the space provided.)

The primary objective of this project is to define biochemical mechanisms involved in eukaryotic DNA synthesis. To approach this problem, we are investigating adeno-associated virus (AAV) and adenovirus DNA replication in in vitro systems. We have now shown that replicating forms of AAV DNA can be generated in vitro utilizing either endogenous or exogenously added templates and cellular polymerase, and that de novo initiation of DNA synthesis can occur in vitro. Two potent inhibitors of AAV DNA synthesis have been purified from KB cells. Among methods used are differential centrifugation, ion exchange and affinity chromatography, gel electrophoresis and isoelectric focusing.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00414-01 LBV

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. The number from one line between the borders.)

JC Virus - A Human Virus that Replicates Efficiently in Brain Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Norman P. Salzman, Ph.D.

| | | | |
|---------|----------------------|----------------------|------------|
| Others: | Shannon Kenney, M.D. | Medical Staff Fellow | LBV, NIAID |
| | V. Natarajan, Ph.D. | Visiting Associate | LBV, NIAID |
| | David Strike, M.D. | Medical Staff Fellow | LBV, NIAID |

COOPERATING UNITS, if any

LAB BRANCH

Laboratory of Biology of Viruses

SECTION

Biochemical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

2.5

PROFESSIONAL

1.5

OTHER

1.0

CHECK APPROPRIATE BOXES:

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

JCV, a human polyoma virus, causes the fatal demyelinating disease progressive multifocal leukoencephalopathy. The virus has an extremely restricted host range and grows well only in human fetal glial cells. The two 98 bp tandem repeats located upstream of the origin of replication in the JC genome have been cloned into the pCAT3M plasmid, which has the bacterial chloramphenicol acetyl transferase gene. The JC tandem repeats, which share significant homology with an 82 nucleotide rat brain specific RNA sequence, were shown to contain stronger enhancer-like activity in fetal glial cells than in other cell types. The narrow host range of JCV is at least partly the result of restricted enhancer activity.



LABORATORY OF CLINICAL INVESTIGATION
1984 ANNUAL REPORT
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SUMMARY OF PROGRAM

Laboratory of Clinical Investigation
October 1, 1983 to September 30, 1984

Michael M. Frank, M.D., Chief of Laboratory
and Clinical Director, NIAID

INTRODUCTION

The year ending in October, 1984 has been a productive one for the Laboratory of Clinical Investigation. Virtually all of our programs are now widely recognized and both senior and junior members of the staff are highly sought after as participants in international meetings and as Board members of the major societies that are associated with their respective areas of research or clinical practice. We continue to have three major programs within the Laboratory: an Allergy Disease Program, an Infectious Disease Program and a Clinical Immunology Program. The Allergy Disease Program is headed by Dr. Michael Kaliner who works in association with Dr. Dean Metcalfe. The Infectious Disease Program consists of the sections of Doctors Gallin, Straus, Bennett, and Quinn and the Clinical Immunology Program consists of the sections of Doctors Frank and Strober. Finally, the Laboratory continues to have a close liaison with the Clinical Parasitology Program under Doctors Neva, Ottesen and Nash. These programs encompass a large spectrum of problems and thus afford investigators unusual flexibility in their research endeavors; they also provide the NIH as a whole with wide ranging coverage of the many clinical problems encountered in infectious diseases and allergy.

The Allergic Diseases Program continues to be focused on research in mast cell biology, mechanisms of histamine action, factors controlling mucus secretion and the pathophysiologic basis of allergic diseases in general. Complimenting these basic interests are clinical interests in asthma, allergic rhinitis, idiopathic anaphylaxis, urticaria and systemic mastocytosis; and the mechanism of gastrointestinal allergy. The Clinical Immunology Program (Clinical Immunology Section) continues to be centered about complement immunology and biochemistry, the nature and role of immune complexes, the factors involved in the lysis of microbes and the definition and characteristics of specific receptors on cells involved in interactions with complement, immune complexes and opsonized particles. In the past year these studies have been extended to the study of the role of complement in host defense against parasitic disease. These basic interests are coupled with clinical interests in hereditary angioedema, hemolytic anemias and the genetic and acquired complement abnormalities. The second arm of the Clinical Immunology Program (Mucosal Immunity Section), continues to be focused on problems in mucosal immunity. These involve studies of the mechanisms controlling IgA synthesis and the nature of immunoregulatory and cytotoxic cells in the GI tract. These basic interests are coupled with clinical interests in malabsorption states and inflammatory bowel disease. The Clinical Immunology Section is headed by Dr. Frank. Ms. Gaither, Drs. Hammer, Brown and Joiner, are members of the group. Drs. Brown and Joiner are both board certified experts in infection disease and participate without program. The Mucosal Immunity Section is headed by Dr. Strober and with Dr. Stephen James, as a member of the group. The latter is a trained

gastroenterologist-hepatologist and thus affords the Laboratory with expertise in gastroenterologic and hepatic diseases.

Within the Infectious Diseases Program, the Bacterial Diseases Section continues to focus on the biology of the phagocytic cell, including basic studies of chemotaxis, cell movement and activation and phagocytic cell biochemistry. Clinical interests of this Section are centered around patients with chronic granulomatous diseases, Chediak-Higashi Syndrome and other granulocyte abnormalities, including patients with hyper-IgE (JOB's) syndrome. This year, Dr. Gallin received a prize from the American Federation of Clinical Research in recognition for outstanding research by a young investigator; this is one of the foremost prizes in the country. Other members of this section include Doctors Bruce Seligmann and Jayasree Nath, a scientist-expert. The Clinical Mycology Section has research interests in the immunologic and pathophysiologic basis of mycotic infections and is considered to be one of the leading groups in this area. Patients with various mycotic infections are studied in connection with this aspect of the Infectious Disease Program. This group is headed by Dr. John Bennett and includes Dr. June Kwon-Chung as a Senior Staff Member. The Medical Virology Section has as its main interest the molecular biology, immunology and treatment of herpes virus infections. This group carries on research on the molecular nature of host resistance and susceptibility to herpes infections as well as the diagnosis and treatment of herpes infections; patients with viral infections constitute the main clinical focus and in this connection the section is at the forefront of treatment of herpes infection, having recently completed a widely recognized evaluation of herpes treatment by acyclovir. The section is headed by Dr. Stephen Straus who has recently been joined by Dr. Jeff Ostrove, a molecular biologist.

As discussed, the members of the various sections join in the care of a variety of patients with allergic, immunologic and infectious diseases. They are aided in this by the Medical Staff Associates whose numbers have grown over the past several years (nine Associates will be joining the Laboratory this year). While training of young physicians is not the primary role of the Laboratory of Clinical Investigation, it has been found over the years that bright, aggressive young physicians contribute enormously to the program by providing new ideas and drive; their training constitutes an obligation to the academic community of considerable importance. Finally, it should be mentioned that both the immunology and infectious diseases aspect of the LCI Program were augmented in the last year by Dr. Thomas Quinn. The latter works both at Johns Hopkins Hospital in the Infectious Diseases Group as well as at NIH in the LCI. Dr. Quinn has become an important research individual in the clinical care of patients with AIDS and has represented the Institute in both Haiti and Zaire. He is helping the Institute establish a program in Zaire for the study of AIDS.

In summary, although the overall structure and scope of the LCI has not changed in the past year, the various programs have matured and broadened and there has been a considerable increase in seniority, expertise and recognition. In support of this latter statement, I can point to the fact that not one, but two of our Senior Staff are currently on the National Board of Allergy: Doctors Kaliner and Strober.

At this point I would like to summarize the research carried out during the past year in each of the components section of the Laboratory.

Allergic Diseases Program

The Allergic Diseases Section continues to be interested in the full spectrum of biological phenomena occurring during the course of immediate hypersensitivity reactions. In basic studies focusing on the nature of histamine receptors, investigators in the section used isolated human lung membranes to obtain evidence that histamine binding to relevant cells occurs via several biochemical mechanisms and there is more than one histamine (H1) receptor on isolated lung membranes; this would explain deviations in linearity that had been obtained in binding curves. In a related project, an analysis of histamine receptors on human lymphocytes was carried out and it was found that a binding of ^3H -pyrilamine to H1 receptors occurs via two distinct classes of interactions indicating that two binding sites exist on human lymphocytes. In other studies vascular permeability following mediator release was addressed. Here, ^3H -dextran of graded size was used to study vascular leaks occurring after cutaneous administration of histamine serotonin or bradykinin. It was found that dextrans with Stokes radii of 140 Å escaped into the interstitium in parallel to that of molecules with Stokes radii of only 40 Å. This study indicates that surprisingly large vascular pores develop following administration of various pharmacologic agents.

These basic studies were accompanied by a number of clinical investigations. Nasal blood flow was measured using an imaginative and sensitive laser-doppler method utilizing a probe placed in the nose; with this technique it was possible to document the effect of various pharmacologic agents on nasal blood flow and to show that atopic patients have a considerably greater response to locally applied methacholine than do normal individuals. A large group of patients with systemic mastocytosis and urticaria were followed and studied in various ways. All patients were found to have elevated plasma histamine levels that do not exhibit diurnal variation. These levels are not effected by antihistamines, chromalyn sodium or prednisone and correlate with the disease severity as assessed by bone scan. Patients have been classified as those with urticaria pigmentosa which slowly progresses over decades and those with mastocytosis, lymphadenopathy and peripheral eosinophilia which is rapidly progressive in the manner of a malignant disease. In an innovative approach to treatment of systemic mastocytosis, IgE-ricin complexes were prepared on the assumption that such complexes could be used to deliver a toxin (ricin) to mast cells preferentially. It was shown that IgE-ricin does in fact cause mast cell death and thus that IgE-toxin conjugates are potentially a feasible method of treatment of systemic mastocytosis. In other studies, patients with idiopathic anaphylaxis have been evaluated. In several cases, such anaphylaxis was linked to sensitivity to endogenous progesterone in that anaphylaxis was induced with subcutaneous injections of the hormone. One such patient was cured by administration of an LH analog which shut down endogenous progesterone production. These studies suggest that a subset of patients with idiopathic anaphylaxis are reacting to endogenous hormones.

Studies of the physiology of mucus secretion are being continued. During this period it was demonstrated that human C3a and C5a cause a dose dependent increase in mucus secretion. Additionally, as part of on-going studies of mast cells it was shown that fibroblasts phagocytize and catabolize mast cell granules. While they are in the extracellular environment, the granules are able to selectively degrade fibronectin via a kinase complexed to the mast cell granule heparin; this fibronectin removing activity in mast cell granules, may explain why mast cell degranulation is not accompanied by scar formation. In association with investigators at Johns Hopkins Hospital a proteoglycan has been identified in cultured basophils obtained from human peripheral blood. This material has been characterized as chondroitin-4-sulfate. The identical proteoglycan has previously been identified in human basophils obtained from patients with chronic myelogenous leukemia. Finally, methods for obtaining mast cells from monkey gastrointestinal tissue have been devised and it was shown that isolated cells respond to pharmacologic agents. These studies hold great promise in allowing investigators to investigate the pharmacology of intestinal mast cells in both health and disease.

Clinical Immunology Program

The Clinical Immunology Section has continued its ongoing studies of immunochemistry of the complement pathway. It was shown that when C5 is cleaved, the C5a cleavage fragment remains associated with the C5 molecule. Only when later components in the complement sequence such as C6 and C7 are available, is C5a release accomplished. The biology of C5a was further explored in studies in which C5a was injected into humans to elucidate the type of cellular reaction elicited. As a result of these studies it is now possible to screen patients with a variety of autoimmune and allergic problems as to abnormalities in their response to injected C5a. In other work, antibodies to the complement membrane attack complex that is responsible for cell lysis has been developed. These antibodies should make it possible to detect in biopsy material the presence of complement related membrane damage lesions.

In continued studies on the lysis of bacteria, the major focus of interest has been on the mechanism of resistance of certain bacteria to lysis. This is of great practical importance because resistant bacteria are able to disseminate and cause clinical illness whereas sensitive bacteria are killed in the circulation and do not cause injury. It has been shown that one mechanism by which surface lipopolysaccharides with long side chains can protect bacteria is by providing sites for complement component attachment that are physically distant from the cell membrane. In contrast, rough bacterial strains or bacterial strains with short chain lipopolysaccharide molecules on their surfaces, provide complement binding sites close to the cell membrane and thereby allow insertion of the attack complex and subsequent lysis. These studies are in parallel with investigations of the mechanism of resistance to serum killing in *Neisseria gonorrhoea*. Here it has been shown that complement lytic attack complexes are associated with specific membrane proteins on bacterial surfaces; in bacteria which are resistant to lysis the complexes are not associated with these membrane

components. This may be an important clue to the mechanism by which *Neisseria* are either lysed or are able to evade the lytic mechanism.

A variety of studies have continued that focus on the role of complement receptors in the pathogenesis of disease. It has been shown that phagocytic cells have a receptor which has the ability to bind C3d. This binding ability is quite weak except in the presence of IgG where binding and phagocytosis are increased enormously. Thus, C3d can allow quantities of IgG, which have no effect alone to produce both binding and phagocytosis of opsonized particles. While the nature of the receptor responsible for C3d binding is not fully defined, it does not appear to be the C3d receptor; data present suggests that both the C3b and the C3bi receptors are capable of facilitating the C3b-IgG binding activity.

Methods have also been developed during this period for purification of C2 in high yields in high functional activity. In addition, studies of the ability of patients with various diseases to phagocytose via the C3b and IgG receptors have begun. In patients with CGD, markedly augmented function of the IgG receptor mediated phagocytosis has been found. This explains why CGD patients do not have an overall phagocytic defect, although they have a decrease in the number of C3b receptors. Finally, data was obtained which suggests that there is enhanced ingestion of particles covered with C3b plus limited amounts of IgG in infected patients, suggesting upregulation of a function of the C3b receptor as well. Studies that allow one to explore the mechanisms by which complement binds to parasitic organisms and thereby controls their disease activity have been initiated; it was determined that a binding site for C3b on *Trypanosoma cruzi* was found; this was associated with a specific protein that was responsible for virtually all the C3b binding. Unusual properties of the C3b IgG complex have been examined and studies which show that this complex can interact with two receptors simultaneously. In addition it has been shown that such complexes are protected from inactivation in the sense that C3b bound to IgG resists activation by factors H and I. Finally IgG has been shown to be a preferential binding site for C3b in the fluid phase; this may explain some of the complement activity in patients with lupus in whom circulating C3b-IgG complexes can be shown to be present. The biological function of such complexes is now under investigation. In studies of the intracellular site of C3b receptor synthesis it has been shown that there one intracellular pool of C3bi receptor which cosediments with specific granules and neutrophils; however, the C3b receptor was shown to also reside in pools which are different from those of the C3bi receptor. Nevertheless, both receptors are upregulated together during cell activation. Functional studies of these receptors will be enhanced with the development of monoclonal antibodies to the C3b receptor and the study of their mechanism of action.

Studies directed at the nature of the immune complexes formed with IgG have continued. It was shown that a neoantigen appears on the IgG molecule following binding to antigen. This neoantigen may permit the development of a new immunoassay for immune complexes that have pathophysiologic relevance. Methods have also been developed which allow for the separation of the adherence and ingestion phases of phagocytosis during the phagocytic process. In addition, studies of the binding of fibronectin to phagocytic cells which enhances phagocytosis via the C3b and IgG receptors have been carried out.

The fibronectin molecule has been fragmented and evidence has been obtained that the fibronectin site responsible for binding occurs in the region of the molecule near the gelatin binding domain. Finally it has been shown that laminin, another protein, is similar to fibronectin in being able to augment the phagocytic process.

In clinical studies carried out on the Clinical Immunology Section, the immunologic analysis of over 100 patients with hereditary angioedema has been completed and it has been shown that about one third have autoimmune phenomena. Analysis of T cell subsets in this disease indicate that patients have elevated numbers of helper cells and normal numbers of suppressor cells. Moreover, patients with marked disease activity as manifested by complement activation have an increased number of helper cells when compared to patients with minimal activation of the complement pathway. In other studies of patients with hereditary angioedema it was found that patients with very low levels of C4, C2 and no functional C1 esterase inhibitor have a protein bound to their C1 inhibitor and that the protein is an antibody.

Mucosal Immunity Section

The Mucosal Immunity Section continued studies on the mechanism controlling IgA B cell differentiation. In previous work it had been shown that B cells bearing surface IgA arise from B cells bearing surface IgM only when the latter are co-cultured with a particular kind of T cell derived from Peyer's patches, a switch T cell. An important characteristic of the switch T cell is that it acts only on IgM B cells and not on IgG B cells; this suggests that switch T cells are class specific and act at a fundamental level of B cell differentiation. One of the important unanswered questions concerning the switch T cell is whether it is an antigen-reactive cell that brings about switch *pari passu* with more conventional helper T cells, i.e., T cells involved in B cell activation, or whether it is an auxiliary cell which is activated by self antigens which appear only during the course of an antigen-driven immune response. To answer this question experiments to determine the capacity of clonal populations of switched T cells to proliferate as a result of exposure to autologous (syngeneic) cells of various sources were studied. It was reasoned that if such proliferation was obtained, switched T cells are autoreactive and are likely to belong to an auxiliary class of cells. It was found that cloned T cells obtained from Peyer's patches and having switch capability proliferated in culture when exposed to activated T cells, activated B cells or activated macrophages. Significantly, this stimulation was blocked by addition to the culture of monoclonal antibodies against appropriate Ia antigens. These studies are strongly supportive of the concept that switch T cells represent cells that are stimulated by activated cells originally stimulated as a result of exposure to antigen. The sequence of events envisaged to occur within the mucosal immune system is therefore as follows: antigen enters Peyer's patches and induces antigen-specific helper T cells and B cells; this results in initial cell proliferation. The proliferating cells now display Ia antigens and can thus stimulate a variety of auxiliary cells such as switch T cells which, in turn, direct B cell differentiation into an IgA pathway. These studies have both theoretical and practical interest, theoretical because they uncover a level of B cell-T cell interaction hitherto not suspected and

practical because manipulation of the switch phenomena can conceivably result in enhancement of immune responses.

In other studies conducted in the Mucosal Immunity Section, investigators continued to define immunoregulatory defects present in patients with inflammatory bowel disease. In these studies, the object was to define the phenotype and the immunoregulatory functional of T cells present in patients on the assumption that the fundamental abnormality present in inflammatory bowel disease is a failure to activate appropriate immunoregulatory T cell subsets in the face of an inflammatory stimulus. In these studies cells obtained from the peripheral circulation as well as the intestinal tissue of patients undergoing surgery for inflammatory bowel disease were examined. In initial studies it was found that the percentage of Leu 2-positive lymphocytes (suppressor/cytotoxic cells) were similar in patients and in the control individuals, when either cells obtained from the peripheral blood or from intestinal sites were examined. Moreover, using techniques in which individual cells were examined for the simultaneous presence of both Leu 2 and Leu 7 antigens (the latter a marker for cells having NK activity), it was found that in this group of patients with severe inflammatory bowel disease these cells were rare both in the peripheral circulation and in the intestinal tissue. This finding contrasts with a previous finding that Leu 2-positive, Leu 7-positive cells are increased in the circulation of patients with mild disease. In further studies it was shown that helper T cell function and suppressor T cell function of cells obtained from both the peripheral circulation and intestinal tissue was similar in patients and controls. In this case, patient and control T cell populations were evaluated for immunoregulatory function using polyclonal activation as a readout system. Here too the findings differed from a previous study of patients with mild disease in that no increase in suppressor T cell function was found. The general conclusion derived from these studies is that patients with severe inflammatory bowel disease do not develop increased suppressor cell activity either in the peripheral blood or at inflammatory sites as might be expected from the inflammatory stimulus present. This lends further credence to the concept that there is inappropriate lack of suppressor cell influence at inflammatory sites in inflammatory bowel disease. To further pursue this concept, studies have been initiated in which primates with induced gastrointestinal inflammation are evaluated as to the pattern of regulatory cells developing both in the peripheral blood and locally at sites of inflammation.

In another ongoing project being undertaken by members of the Mucosal Immunity Section, the autologous mixed lymphocyte reaction is being studied. The autologous mixed lymphocyte reaction is the proliferative response of T cells resulting from their exposure to Ia antigens on autologous B cells and macrophages. It was previously shown that this reaction leads to the elaboration of immunoregulatory cells and is thus of interest to the study of immunologic diseases. During the current period, studies were conducted to determine the immunoregulatory activity of autoreactive T cell lines and clones. Specifically, a cloned T cell line was obtained from cells repeatedly stimulated *in vitro* with autologous B cells and macrophages. Interestingly, the cloned T cells obtained bore the OKT4 antigen, that is, they bore an antigen usually associated with helper/inducer function. In detailed studies of this cell line and clone, it was found that the cells

exerted their activity directly on B cells and elaborated a suppressor factor. These studies indicate that suppressor cells generated by the autologous mixed lymphocyte reaction may have a particular role in the pathogenesis of autoimmunity, where B cell hyperactivity is a regular occurrence.

Infectious Diseases Program

In the Bacterial Diseases Section a number of aspects of phagocytic cell biology were successfully explored both in normals and in patients with defective chemotaxis, degranulation and oxidative metabolism. In studies designed to elucidate neutrophil degranulation, investigators in this section have developed an ingenious method of reproducing in vitro an essential step in the degranulation process-fusion of granules with the inside of the plasma membrane. In this method, latex beads are fed to organelle-depleted neutrophils (cytoplasts) and the internalized beads, now coated with inside-out plasma membranes are re-isolated and are incubated with isolated granules. It is observed that the coated beads and isolated granules undergo fusion in vitro thus providing a model for the fusion of granules with the inside of plasma membranes within the cell. This in vitro fusion reaction can be studied using biochemical and immunochemical techniques. For instance, members of the section have developed monoclonal antibodies which are specific for proteins on the inner surface of the plasma membrane which can be used to probe the fusion reaction. In other studies done in collaboration with the Clinical Immunology Section, it was shown that the intracellular pool of C3bi receptors within neutrophils co-sediments on sucrose gradients with specific granules. In addition, the C3bi receptors are translocated to the plasma membrane upon cell activation. These findings were corroborated by study of a patient lacking specific granules who was shown to also lack C3bi receptors. In studies designed to define neutrophil subsets, monoclonal antibodies with specificity for human peripheral neutrophils were prepared and characterized. In these studies, a monoclonal antibody was obtained which identifies the population of neutrophils which responds to the chemoattractant f-met-leu-phe as well as other stimulants. A second population was found which does not bind the antibody and which responds to chemo attractant only after priming in vitro. These monoclonal antibody studies therefore demonstrate the presence of two types of neutrophils in the circulation. In studies of neutrophils from a biochemical standpoint, it was found that a variety of stimuli including f-met leu-phe causes neutrophil tubulin tyrosinolation of normal cells and not of cells of patients with chronic granulomatous disease. In related studies of the subcellular localization of tubulin tyrosinolation, an endogenous ligase was detected in the azurophil granules of neutrophils; this is the first demonstration of intracellular organelle-associated ligase in its functional form.

These basic studies of neutrophil function were complimented by investigation of patients with neutrophil disorders. Chronic granulomatous disease (CGD) heterozygotes were analyzed as to the variability the percentage of cells expressing the CGD defect. The data obtained, support for the thesis that 1) neutrophil myelopoiesis is characterized by the concurrent maturation of many clones and that 2) the Lyonization (inactivation of the X chromosome) occurs

at the ninth cell division of the precursor cell destined for myeloid maturation. In other clinical studies, members of the section have shown that EB virus transformed B cell lines derived from B cells in the peripheral blood of patients with CGD and Chediak-Higashi Syndrome are characterized by the very same biochemical defects present in the neutrophils in these patients. This finding is a technical breakthrough because it will now be possible to study large numbers of cells obtained from patients with these disease states. An interesting patient with persistent leukocytosis and recurrent infections who had abnormal neutrophil adhesion and aggregation but normal degranulation was extensively studied. It was found that the patient's cells had a deficiency in the C3bi receptors and did not bind the monoclonal antibody OKM1. This patient's defect therefore suggests that the OKM1 antigen may be necessary for neutrophil adhesion and aggregation. In studies designed to uncover disease heterogeneity in CGD, *in vitro* cell fusions were carried out to see if fused cells could be obtained that were biochemically repaired, i.e., if the fusion partners obtained from different patients had non-overlapping defects. Cytoplasts exhibiting abnormal oxygen metabolism were prepared from different patients and fused in the presence of polyethylene glycol. It was found that (at least in some cases) fused cells could indeed be obtained which had normal oxygen metabolism; this indicates that CGD is probably a heterogeneous group of disease with defects affecting different parts of the oxidative pathway. In studies designed to define the biochemical defects in chronic granulomatous disease, patients with the disease were analyzed for the presence of a cytochrome b deficiency. It was found that approximately 9 of 25 patients had such a deficiency and thus this enzyme defect may play a role in the significant number of patients. Finally, in studies of patients with hypergammaglobulin E and recurrent infection (Job's syndrome) it was shown that patients had a severe defect in IgA antistaphylococcal aureas antibody synthesis which was manifest in both serum and saliva. This unexpected IgA defect may account for some of the difficulties patients have with infections and may provide an important clue as to the immunopathogenesis of the disease in that the excessive IgE antibody production found in patients may be due to a block in the IgA pathway.

Medical Virology Section

The Medical Virology Section has continued to pursue studies centered on the pathogenesis, molecular biology, immunology, and therapy of human herpes virus infections. During the past year approximately 60 new patients have been identified and examined at the NIH with a wide variety of herpes virus infections. Selected individuals have been studied serially to assess viral shedding during the course of natural and treated infections. Studies of the capacity of acyclovir to suppress herpes simplex infection have continued, focusing during this period on immunodeficient individuals. Approximately 100 patient-months of chronic anti-viral suppressive therapy of immunodeficient individuals have been completed without adverse effects. Resistant strains have been identified more frequently in patients whose infection cannot be suppressed and have emerged in patients who develop recurrent infection while on therapy. Placebo-controlled trials of chronic oral acyclovir therapy were continued this year in 40 immunologically normal patients. In one trial it was shown that viral shedding was frequent in the

absence of treatment but dramatically reduced in the presence of treatment. In another trial, intermittent therapy was shown not to be as successful as daily treatment.

These studies have been complemented by studies of the molecular biology of varicella zoster virus (VZV) infections. A library of varicella zoster DNA restriction fragments cloned in λ phage and plasmid vectors was developed and the clones used in a rapid dot-blot hybridization assay for diagnosis of infection. This assay is superior to and could supplement conventional culture techniques. In other studies using the cloned VZV DNA fragment, infected cells were probed for virus-specific transcripts. Such transcripts were in fact found and related by earlier studies to the DNA encoding a major envelope protein of the virus. This molecular approach is being used to examine acyclovir-resistant strains of VZV; in particular, the DNA fragments from such strains are being cloned to map the locus of genes responsible for drug resistance. This molecular approach has also been used to analyze an immunodeficient patient who experienced a recurrent zoster infection 5 months after a primary infection. It was found that both infections were due to the same virus, thereby providing the first definitive support for the suggestion that zoster is due to reactivation of latent varicella virus. Finally, extensive studies of herpes virus infection in AIDS patients have been accomplished. Analysis of nearly 100 specimens obtained from 30 AIDS patients indicate that herpes virus infection are clinically apparent in about 20% of patients, a frequency less than that encountered for CMV and EBV (which occur in almost 100% of patients). However, herpes virus infection in AIDS patients was unusually persistent and severe. The infections were suppressed with acyclovir.

In another series of studies, members of the Medical Virology Section have studied enteric adenoviruses (EAdVs), a group of related viruses causing gastroenteritis in infants. Methods for growing the virus have been developed in the Section and efforts to characterize these viruses by molecular techniques are now well advanced. The latter includes study of the virus by endonuclease cleavage analysis, blot hybridizations and molecular cloning in plasmid vectors. In two prototype viral strains, cloned DNA fragments unique to EAdVs have been obtained and have been used to develop a rapid test for the occurrence of virus in stool specimens. Efforts to determine the basis of host restriction and tropism of the virus have been initiated. First, viruses have been purified and used in binding studies to assess the ability of EAdVs to initiate infection in permissive and non-permissive cell lines. Second, early mRNA synthesis, studied with RNA/DNA dot-blots or Northern hybridization, have been used to detect differences in virus growth patterns in different cell lines.

Clinical Mycology Section

The Clinical Mycology Section continued studies centered on the diagnosis of systemic candidiasis. A latex agglutination test has been devised which has been useful in the rapid diagnosis of acute life threatening mycoses. Using the strategy of pretreating serum with proteases and heat, the sensitivity of the test was greatly increased. Candida antigen was detected in about 80% of patients with severe disseminated candida infections, but only rarely in

patients with localized disease or controls. In systemic candidiasis, the most common fatal mycoses, it was found that the antigen was present in high molecular weight immune complexes, which upon dissociation was shown to contain mannan of a probable molecular weight of 800,000 Kd. In all, this test will probably be of considerable value in the identification of severe candida infections.

The members of the Clinical Mycology Section also continued their studies of humoral immunity in cryptococcosis. Specifically, they investigated the lack of responsiveness to cryptococcal polysaccharide in patients with cured cryptococcal infection. In the relevant studies, patients and controls were immunized with cryptococcal polysaccharide antigen and seven days later their peripheral cells were examined for capacity to secrete anti-cryptococcal polysaccharide antibodies *in vitro*. It was found that patients with cured cryptococcal infections had circulating cells that made only IgA cryptococcal antibody and not IgG or IgM antibody, whereas controls made antibodies belonging to all classes. In contrast, if a control antigen was used, ie pneumococcal polysaccharide, both patients and controls had cells after seven days which secreted all classes of antibody. In some patients the IgA responses were quite significant and were accompanied by the presence of IgA anti-cryptococcal polysaccharide antibody in the serum. Since the lack of IgM and IgG responses were also observed when purified B cell populations were examined, the defect cannot be ascribed entirely to a suppressor T cell. In separate studies, it was established with the use of a rosetting technique that immunization of control individuals is followed by an increased number of circulating cells binding cryptococcal polysaccharide antigen. In contrast, immunization of patients with cured cryptococcosis is not followed by the appearance of cryptococcal polysaccharide binding cells. This finding suggests that patients with cured cryptococcosis have experienced clonal deletion of cryptococcal polysaccharide antigen-reactive cells. In all, these studies uncover what may be a very important deviation in the way patients who have had disseminated cryptococcal infection respond to subsequent antigen stimulation. In addition, they suggest that the IgA response may have a special role in the immunity developed in patients with cryptococcal infection.

Clinical Parasitology Section

The Clinical Parasitology Section is continuing to carry out a wide variety of studies into many aspects of parasitic infection. During the past year studies have been conducted both within the confines of the Clinical Center as well as in clinical units located in the field. In studies of patients with lymphatic filariasis attention was focused on mechanisms regulating IgE synthesis induced by filarial infection. It was determined that patients contain B cells in their circulation which spontaneously produce IgE. In a related study filarial antigen-specific lines and clones have been developed and factors derived from such lines and clones which favor IgE B cells have been identified. IgG anti-filarial antibodies which inhibit immediate hypersensitivity have been detected in the circulation of patients and have been shown to correlate the clinical findings in patients. Since IgG4 is elevated in patients with filariasis and since IgG4 and IgE responses are qualitatively similar, IgG4 is being evaluated as a possible blocking

antibody for IgE mediated reactions. In other work, filarial antigens have been detected in the circulation of patients using an immunoradiometric and immunoblotting technique. In addition, patients in Madras, India with tropical eosinophilia have been subjected to bronchial lavage in order to assess local immunologic responses. In these studies, the patients were found to have abnormal alveolar cellular constituents characterized by a predominance of eosinophils and macrophages. Currently, the lavage fluids are being further analysed to better understand the locally destructive inflammatory mediators responsible for the alveolitis present in tropical eosinophilia.

In studies of onchocerciasis, analysis of the side effects of treatment of the disease with diethylcarbamazine (the Mazzotti reaction) is being conducted. It was found that the reaction is accompanied by a decrease serum complement level as well as activation of immediate hypersensitivity mechanisms. These facts suggest that the reaction can be treated with measures that counteract the effects of inflammatory mediators released from mast cells. In studies of loiasis, a trial of prophylactic diethylcarbamazine in Peace Corp volunteers going to African countries was initiated. Rates of seroconversion and acquisition of clinical disease will be followed. In studies of schistosomiasis humoral antibody responses present in IgG and IgA classes are being measured using western blot techniques in association with worm and egg antigens. So far these studies have revealed a wide range of individual responses and that antibody responses are indicative of acute disease. In studies of strongyloidiasis, additional analyses of the anti-worm antibody titers detected by ELISA technique are being recorded. A followup of patients who have treated for strongyloidiasis show a definite downward trend in antibody titers following treatment.

In studies of giardiasis improved techniques for the speciation of the organism have been devised. Comparison of the organism by various techniques including DNA endonuclease restriction enzyme analysis as well as by more conventional biochemical techniques are disclosing large differences among the organisms isolated from various individuals. The significance of this finding have yet to be explored. Finally, in studies of leishmaniasis, organisms are being characterized as to their biological behavior in mice. In addition, it was found that diffuse cutaneous leishmaniasis in Ethiopian patients differs from the regular cutaneous disease in that cells from diffuse disease patients have a deficiency in IL-2 and interferon production upon stimulation with the leishmanial organisms. Leishmanial isolates from diffuse cutaneous and regular cutaneous leishmaniasis will now be compared as to their type and other biologic properties.

Acute Infectious Disease Unit: (Johns Hopkins Hospital) Studies of the clearance of Igb sensitized autologous erythrocytes from the circulation of patients with AIDS have been conducted. A marked defect in clearance was observed in a high percentage of patients with active AIDS and in almost all patients with episodes of disseminated infection. The results suggest that such a defect may permit dissemination of the infectious agents responsible for superinfection and death.

Conclusion

The studies outlined above represent the broad-gaged yet intense research effort in the area of immunology, allergy and infectious disease. Each component of the LCI program is engaged in problems that address both basic and clinical aspects of various diseased states. Investigators in the Laboratory are widely recognized, active scientists who are also playing a major role in patient care and the teaching of staff associates.

HONORS AND AWARDS

This year Dr. John Gallin was awarded a prize by the American Federation for clinical research given annually for the best young clinical investigator in America. This is the second year that this prize has been awarded and receipt of the award represents a major achievement. Dr. Stephen Straus was awarded a Commendation medal from the Public Health Service. Dr. Frank received the McLaughlin Prize Medal from the University of Texas. Two members of the staff have currently been elected to the board in Allergy and Clinical Immunology, Dr. Warren Strober and Dr. Michael Kaliner. Dr. Kaliner will be chairman of the board from 1984 to 1986. Dr. Kaliner is also president elect of the American Thoracic Society Scientific Assembly on Allergy and Immunology and is member of the Program Committee of the American Association of Immunologists. Many editorial board positions are filled by members of the Senior Scientific Staff. Dr. Frank continues as a editor of Reviews in Infectious Disease and Clinical Immunology Reviews. He serves as editor of the Journal of Clinical Immunology and Journal of Allergy and Clinical Immunology as well as Proceedings of the Society for Experimental Biology and Medicine. Both Dr. Metcalfe and Dr. Kaliner are also members of the editorial board of the Journal of Allergy and Clinical Immunology. Dr. Gallin is an associate editor of the Journal of Immunology and serves on the editorial board of the Journal of Clinical Investigation, Infection, and Immunity, the Journal of Leucocyte Biology, Cellular Immunology and Advances in Inflammation Research. He serves as an editor of Advances in Host Defense Mechanisms. Dr. Metcalfe has asked to serve on the committee of adverse reactions to food of the American Academy of Allergy as well as a number of other committees for the academy. Dr. Bennett serves on the editorial board of Antimicrobial Agents in Chemotherapy, the Journal of Clinical Microbiology and Pediatrics Infectious Disease as well as the editorial editor of the Journal Sabouraudia and Microbiological Sciences and participated in the International Mycological Congress in Tokyo as well as a number of other major congresses.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00043-19 LCI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunology and Chemotherapy of Systemic Mycoses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Dr. John E. Bennett, Head, Clinical Mycology Section, LCI, NIAD

COOPERATING UNITS (if any)

*Johnsie W. Bailey, Bureau of Divices, FDA, Silver Spring, MD
Dr. Carmelita Tuazon, George Washington University, Washington, D.C.
Dr. Coorstian Brass, State University of New York, Buffalo*

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Mycology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, N.I.H. Bethesda, MD

TOTAL MAN-YEARS:

4.4

PROFESSIONAL:

2.4

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A latex agglutination test was highly successful in detecting Candida antigen in serum from patients with severe disseminated candidiasis, provided that the antigen was first dissociated from high molecular weight complexes using protease and heat. Of 21 patients with disseminated candidiasis, 81% had antigen in at least one serum, titers ranging 1:4 to 1:64. Serum from patients with less severe clinical forms of candidiasis were infrequently positive. False positives were rare. The test appears promising as an aid to rapid diagnosis.

Patients cured from cryptococcosis but otherwise normal are known from our previous work to be selectively tolerant to immunization by cryptococcal polysaccharide. This tolerance was recently shown in peripheral blood cells cultured in vitro, even purified B cells, free of potential T cell suppression. A subset of these patients was not completely tolerant but responded to immunization by anticryptococcal antibody only of the IgA class.

Peripheral blood monocytes from patients with acquired immune deficiency syndrome (AIDS) had unimpaired ability to kill fungal cells from Aspergillus, Cryptococcus and Thermoascus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00045-16 LCI

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Studies on Interaction of Antibody and Complement on Production of Immune Damage

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)
PI: Michael M. Frank, M.D. Head, Clinical Immunology Section LCI/NIAID
and Chief
Others: Carl H. Hammer, Ph.D. Senior Investigator LCI/NIAID
Thelma Gaither Research Biologist LCI/NIAID
Lois Renfer Chemist LCI/NIAID
Chaim Brickman, M.D. Medical Staff Fellow LCI/NIAID
Kathleen Melez, M.D. Guest Scientist LCI/NIAID
Irma Vargas Biologist LCI/NIAID

COOPERATING UNITS (if any)
Neil Young, M.D. IR/CHB/NHI
Jeffrey Moore IR/CHB/NHI

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Clinical Immunology Section

INSTITUTE AND LOCATION
National Institute of Allergy and Infectious Diseases

| | | | | | |
|-----------------|-----|--------------|-----|-------|-----|
| TOTAL MAN-YEARS | 5.2 | PROFESSIONAL | 3.2 | OTHER | 2.0 |
|-----------------|-----|--------------|-----|-------|-----|

CHECK APPROPRIATE BOXES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)
Immunologic analysis of 156 patients with hereditary angioedema has been completed and it has been shown that about a third have autoimmune manifestations. However, in the vast majority of patients, these manifestations are clinically minor. Analysis of T cell subsets indicate that patients with hereditary angioedema have elevated numbers of helper cells and normal numbers of suppressor cells. The origin of the increase in helper cells is not known but there is a clear correlation between complement activation and helper cell number. Thus, patients with marked disease activity as manifested by complement activation have an increased number of helper cells when compared to patients with minimal activation of complement. In other studies, efforts are underway to further analyze a series of patients with acquired angioedema. A number of these patients have been followed for up to a decade at the National Institutes of Health. These patients tend to have 0 levels of C4, C2 and no functional C1 esterase inhibitor. Careful analysis of serum suggests that they have a protein bound to the C1 inhibitor and that such protein is an antibody. Another major interest in our laboratory has been the expression of complement receptors on phagocytic cells. We had previously shown that peripheral blood monocytes possess the receptor for the complement fragment C3d. In the past year, we have shown that C3d coated particles can be phagocytosed in the presence of particle-bound IgG and that C3d causes marked augmentation of IgG mediated phagocytosis. At present, the identity of the receptor responsible for the binding of the C3d coated particles is not clear. It does not appear to be the same C3d receptor which exists on lymphocytes and may simply represent combined activity of the C3bi and C3b receptors respectively.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00047-15 LCI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Studies of Patients with Known or Suspected Parasitic Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: E. A. Ottesen, Head, Clinical Parasitology LCI/LPD, NIAID
Section

Others: See Next Page

COOPERATING UNITS (if any)

See Next Page

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Parasitology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

3.0

PROFESSIONAL

3.0

OTHER

0.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of these studies is to increase understanding of the diagnostic, therapeutic and pathogenicity aspects of parasitic infections.

Reliable serodiagnostic assays have been developed for filariasis (detection of circulating antigen), strongyloides (detection of IgG antibodies) and schistosomiasis (detection of IgG and IgM antibodies). Under development are diagnostic assays for strongyloides (skin test), cryptosporidia (serum antibody) and schistofibrotic liver complication of schistosomiasis (by ultrasonography).

Therapeutic studies indicate that while heat treatment is effective for lesions of patients with disseminated cutaneous leishmaniasis (DCL) of the Americas, it is ineffective in Ethiopian DCL. Long-term studies of diethylcarbamazine (DEC) used prophylactically for loiasis are underway in Africa.

The pathology of parasitic infections results from both the patients' immune responses to the parasite and the pathogenic potential of the parasite itself. For helminth infections (filariasis, schistosomiasis, onchocerciasis, loiasis, strongyloidiasis) cellular and humoral (especially IgE and the subclasses of IgG) immune responses and their specific regulatory mechanisms have been characterized. Additionally, bronchial lavage in patients with tropical eosinophilia has indicated the presence of an eosinophil-macrophage alveolitis; the locally destructive inflammatory mediators are being characterized. Studies of the violent side effects of treatment with DEC in patients with onchocerciasis indicate important roles for complement and immediate hypersensitivity immune mechanisms in initiating these reactions. Work on pathogenicity in protozoal infections (giardiasis, leishmaniasis) has focused primarily on strain differences among the parasites and their roles in determining the character of the pathology and the clinical response of these patients to treatment. Additionally, the importance and mechanisms of immunologic anergy in patients with DCL are under study.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00048-14 LCI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Pathophysiology of Autoimmune Hemolytic Anemia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Michael M. Frank, M.D. Head, Clinical Immunology Section LCI/NIAID
and Chief

COOPERATING UNITS (if any)

Thomas Lawley, M.D., DB/NCI Paul Plotz, M.D., NIADK
Thomas Quinn, M.D., LCI/NIAID
Brad Bender, M.D., Instructor of Medicine, JHU

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

1.5

PROFESSIONAL

1.5

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Studies of the reticuloendothelial system and the clearance of particles from the circulation have continued. As detailed in Dr. Quinn's outline, a specific defect has been discovered in patients with AIDS syndrome. Such a defect may predispose these patients for dissemination of intracellular infection as is noted to occur in this group. This is felt to be a secondary defect rather than primary to development of the AIDS syndrome.

Last year we reported striking changes in mononuclear phagocyte Fc receptor expression induced by pharmacologic glucocorticoid therapy. Studies of similarly treated normal volunteers were carried out to characterize changes in in vitro clearance. In each individual studied, subtle slowing of Fc receptor mediated clearance was demonstrated following clinically relevant glucocorticoid doses.

Studies are in progress to evaluate possible sex differences in Fc-receptor mediated clearance in humans (as a possible reflection of previously demonstrated quantitative differences in receptor expression on monocytes from male and female subjects). In addition, the existence of familial abnormalities in clearance is being sought in the families of patients with systemic lupus erythematosus.

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZOI AI 00057-11 LCI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Basic Studies on pathogenic fungi

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

K.J. Kwon-Chung, Research Microbiologist, LCI/NIAID

COOPERATING UNITS (if any)

T. Folks (NIAID)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Mycology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases

TOTAL MAN-YEARS:

2.5

PROFESSIONAL:

1.0

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Topics of current studies include: 1) characterization of unusual strains of Thermoascus crustaceus isolated from the monocytes of AIDS patients, 2) isolation of an isogenic sets of C. albicans including extracellular proteinase positive wild type, negative mutant and positive revertants to compare their virulence for mice, and 3) determination of geographic distribution of the two varieties of C. neoformans. Three isolates of Thermoascus crustaceus were isolated from monocyte cultures of patients suffering from AIDS. The three isolates were atypical in that the ascospores had smooth wall while those of the typical T. crustaceus were echinulate. The atypical T. crustaceus has not been reported from clinical specimens. The mycelial extracts of these AIDS isolates produced immunosuppressive material as determined by lymphocyte bioassays. When the spores of T. crustaceus were injected into mice intraperitoneally, the spores did not germinate but survived in mouse tissue for three to six weeks. Extracellular proteinase has been implicated as a virulence factor in Candida albicans. We were successful in isolating isogenic sets of strains including proteinase positive wild type, negative mutant and the positive revertant originating from one isolate. These isolates will be tested for their virulence for mice. The epidemiological differences between the two varieties of Cryptococcus neoformans were studied using 725 clinical isolates obtained from various parts of the world. It showed that the C. neoformans var. gattii (serotype B.C) is prevalent only in tropical and subtropical regions of the world while C. neoformans var. neoformans is prevalent throughout the world. C. neoformans var. gattii is not found in Europe and Japan. In the United States, C. neoformans var. gattii is prevalent only in Southern California and is infrequently found from the remaining part of the U.S.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00058-10 LCI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Pathogenesis and Chemotherapy of Herpesvirus Infections in Man

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: S.E. Straus Senior Investigator, LCI, NIAID

| | |
|-------------------------------|-------------------------------------|
| OTHER: H. Takiff (until 4/84) | Medical Staff Associate, LCI, NIAID |
| M. Seidlin (until 6/84) | Medical Staff Associate, LCI, NIAID |
| J. Rooney | Medical Staff Associate, LCI, NIAID |
| J. Felsner | Medical Staff Associate, LCI, NIAID |
| J. Ostrove | Senior Staff Fellow LCI, NIAID |

COOPERATING UNITS (if any)

J. Hay (USUHS), W. Ruyechan (USUHS), R. Whitley (Birmingham, Alabama)
 H. Masur (CC, NIH), G. Quinnan (BOB/FDA), G. Tosato, M. Blaese (MET/NCI)
 S. Nosinoff-Lehrman (Burroughs Wellcome Company)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

3.5

PROFESSIONAL

2.0

OTHER

1.5

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

The pathogenesis, molecular biology, immunology, natural history, and therapy of human herpesvirus infections are being investigated. Immunocompetent and immunodeficient patients, including those with AIDS, who possess a wide range of herpesvirus infections have been identified and studied. During the past year we have extended our studies of chronic EBV infection, having identified a series of immunologic abnormalities in that syndrome; we have continued to study acyclovir suppression for frequently recurring or chronic herpes simplex infections, with encouraging results.

Our major focus on the study of the molecular biology and latency of varicella zoster virus (VZV) DNA has continued. We have proven reactivation of latent VZV by restriction endonuclease analyses of isolates recovered from separate episodes of infection in the same individual. Recombinant clones were successfully used for rapid diagnosis of varicella and zoster infection. This year we have begun studies of the genetic expression of VZV, with at least preliminary characterization and mapping of a series of major and minor viral transcripts from the right hand end of the viral genome. During the coming year we plan to initiate marker rescue studies to map the genetic loci associated with VZV resistance to antiviral drugs.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00154-09 LCI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Events in Immediate Hypersensitivity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---------|----------------------|---------------------------------|-----------|
| PI: | Michael A. Kaliner | Head, Allergic Diseases Section | LCI/NIAID |
| Others: | Thomas Casale | Staff Fellow | LCI/NIAID |
| | Thomas Keahy | Staff Fellow | LCI/NIAID |
| | Howard Druce | Visiting Associate | LCI/NIAID |
| | Howard Boltansky | Staff Fellow | LCI/NIAID |
| | Susan Wescott | Biologist | LCI/NIAID |
| | Paul Kauffman | Medical Technologist | LCI/NIAID |
| | Rosemary Pelliciotto | Medical Technologist | LCI/NIAID |

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Allergic Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

7

PROFESSIONAL:

4

OTHER:

3

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects
 ☒ (b) Human tissues
 ☐ (c) Neither
- ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our analysis of events in immediate hypersensitivity focuses on human and animal models of allergic responses, mechanisms of mediator action, and pharmacologic approaches to allergic diseases. The areas under investigation include asthma, allergic rhinitis, anaphylaxis, urticaria and mastocytosis. Histamine, one of the major mediators released by human lung, was shown to interact with 3 distinct H1 receptors on isolated human lung membranes. Similarly, histamine may react with two H1 receptors on purified human lymphocytes. It appears that infusions of dopamine may inhibit histamine skin reactivity; therefore, skin tests on patients receiving dopamine are invalid. The mechanism for narcotic induced mast cell degranulation was examined and found to involve both specific opiate receptors as well as receptor-independent mechanisms. Vascular permeability can be examined by measuring the leak of radiolabeled IgG or well-defined dextrans. The use of dextran permits analysis of vascular leakage over a long period and will facilitate study of microvascular events during cutaneous inflammatory reactions. Nasal allergic reactions involve rhinorrhea; therefore, a model for the study of nasal secretions in humans in vivo was developed. Methacholine acting on muscarinic receptors stimulates mucus secretion and atopy is hyperresponsive compared to normal controls. As mast cells are the cellular midus of allergic reactions, depletion of mast cells might be a powerful method of therapy. Strategies for mast cell killing include IgE-ricin and IgE-ricin A chain. Both methods cause mast cell death.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00155-09 LCI

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Phagocytic Cell Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

| | | | |
|---------|---------------------------------|---------------------------|-----------|
| PI: | John I. Gallin, M.D. | Chief, Bacterial Diseases | LCI/NIAID |
| Others: | E. Stephen Buescher, M.D. | Medical Staff Fellow | LCI/NIAID |
| | Robert L. Roberts, M.D., Ph.D. | Medical Staff Fellow | LCI/NIAID |
| | Bruce E. Seligmann, Ph.D. | Senior Staff Fellow | LCI/NIAID |
| | Jayasree Nath, Ph.D. | Expert | LCI/NIAID |
| | Yoichiroh Ohno, M.D., Ph.D. | Visiting Associate | LCI/NIAID |
| | Stephen C. Dreskin, M.D., Ph.D. | Medical Staff Fellow | LCI/NIAID |

COOPERATING UNITS (if any)

| | |
|------------------------|-----------|
| David W. Alling, M.D. | OSD/NIAID |
| Anthony S. Fauci, M.D. | LIR/NIAID |
| David C. Volkman, M.D. | LIR/NIAID |
| Michael M. Frank, M.D. | LCI/NIAID |

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Bacterial Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

8

PROFESSIONAL:

5

OTHER

3

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Several aspects of phagocyte biology have been studied using neutrophils (PMN) and monocytes from normal subjects and patients with defective phagocyte chemotaxis, degranulation and oxidative metabolism. Studies of the myelopoiesis and timing of Lyonzation were performed using females heterozygote for chronic granulomatous disease. The data indicate neutrophil myelopoiesis is by a polyclonal system and that Lyonzation occurs at about the ninth myeloid cell division. In other studies of intracellular pools of PMN receptors evidence was obtained indicating that the intracellular pool of C3bi receptors cosediments with specific granules on sucrose gradients. Like our previous studies with the fmet-leu-phe receptors, the C3bi receptors get translocated to the plasma membrane with cell activation. A patient lacking the C3bi receptor was shown to have severe defects of neutrophil adherence and aggregation.

EB virus transformed B cells were shown to produce superoxide in response to phorbol myristate acetate making it possible to store normal or patient cells with this capacity. Further studies of neutrophil degranulation resulted in the development of a new cell free model to study granule fusion with the inside of the plasma membrane. Monoclonal antibodies that recognize the inside, but not the outside of the plasma membrane have been made.

In other investigations a new technique was developed for purification of normal eosinophils. This technique, which employs differential responsiveness of eosinophils and neutrophils to fmet-leu-phe, makes it possible to obtain 95-100% pure eosinophils from normal subjects.

Studies of immunoglobulins from patients with the Hyperimmunoglobulin E-Recurrent Infection (Job's) syndrome indicate a deficiency of antistaphylococcal IgA in this disease. This helps explain the propensity of these patients to staphylococcal infection.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00189-05 LCI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical and Biochemical Studies of Human Enteral Adenovirus Infections

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Stephen E. Straus, Senior Investigator, LCI, NIAID

OTHER: H. Takiff (until 4/20/84) Medical Staff Fellow LCI, NIAID

J. Rooney Medical Staff Fellow LCI, NIAID

COOPERATING UNITS (if any)

C. Brandt and W. Rodriguez (Children's Hospital, D.C.), H.S. Ginsberg
(Columbia Univ.), R. Yolken (Johns Hopkins), J. Esparza (Caracas, Venezuela)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.25

PROFESSIONAL:

1.0

OTHER:

0.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Enteral adenoviruses (EAd) comprise two related serogroups of viruses which appear to cause gastroenteritis in infants. Studies of these viruses and the diseases they produce were hampered until recently by an inability to grow them in tissue culture. Using a method we have developed for growing these viruses, numerous isolates from around the world have been studied. EAd DNA fragments have been cloned in plasmid vectors, and DNA restriction maps of serogroup F and G strains have been completed. Analyses of the genomic structures of these viruses demonstrated partial sequence homologies to conventional adenoviruses. Recombinant clones derived from areas showing little or no homology to non-EAd have been used to develop a highly sensitive and specific dot-blot assay for EAd in clinical specimens. Our current efforts include further molecular epidemiologic studies, characterization of EAd and Ad binding to specific tissues, and mapping of early viral MRNAs.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00192-06 LCI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on Immediate Hypersensitivity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Dean D. Metcalfe, M.D.

Senior Clinical Investigator

LCI/NIAID

Others: Fred M. Atkins, M.D.

Medical Staff Fellow

LCI/NIAID

COOPERATING UNITS (if any)

Laboratory of Vision Research, National Eye Institute (I. Gery), The University of Washington (C.W. Henderson and S. Klebanoff), and Johns Hopkins University (T. Ishizaka)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Allergic Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

2.25

PROFESSIONAL

1.25

OTHER

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☒ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Fibroblasts and endothelial cells both are capable of the phagocytosis and degradation of mast cell granules as demonstrated by microscopy and by the use of radiolabeled mast cell granules. The degradation of heparin proteoglycan follows the exposure of this molecule to reactive radicals formed as a consequence of the respiratory burst. This process is rapid and results in heparin proteoglycan products that appear similar to the heparin fragments found in commercial preparations of heparin.

Mast cell granules rapidly degrade extracellular fibronectin. While this degradation is probably due to chymase, mast cell granules are particularly efficient at cleaving fibronectin. This may represent a major extracellular function of mast cell granules and influence repair mechanisms within connective tissues.

Basophils from human cord blood synthesize chondroitin 4-sulfate, a molecule previously identified in mature human basophils.

The induction of autoimmune uveitis is associated with choroidal mast cell numbers in a rat model of this disease.

Proteoglycan heparin is rapidly degraded following exposure to reactive radicals formed during the respiratory burst. The products have an approximate molecular weight of 12,000, which is similar to the size of heparins in commercial preparations. This observation may explain how phagocytic cells are able to rapidly degrade proteoglycan heparin and mast cell granules.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00249-03 LCI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Pathogenesis, Diagnosis, and Treatment of Systemic Mast Cell Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Dean D. Metcalfe, M.D.

Senior Clinical Investigator

LCI/NIAID

Others: William Meggs, M.D., Ph.D.

Medical Staff Fellow

LCI/NIAID

COOPERATING UNITS (if any)

Digestive Diseases Branch, National Institute of Arthritis, Diabetes, and
Digestive and Kidney Diseases (Jay Cherner)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Allergic Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.25

PROFESSIONAL:

1.25

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

All patients with systemic mastocytosis have elevated plasma histamine levels. These plasma histamines remain consistently elevated over a 24-hour period. No diurnal variation was noted. Patients with urticaria pigmentosa have normal or slightly elevated plasma histamines, while patients with idiopathic anaphylaxis show elevations in plasma histamine during attacks of anaphylaxis. Plasma histamine elevations in subjects with systemic mastocytosis are not affected by antihistamines (chlorpheniramine and cimetadine), cromolyn sodium, or prednisone. Plasma histamines correlate with disease severity as assessed by bone scan.

Two forms of mastocytosis have been documented. Ninety percent of our patients have disease presenting initially as urticaria pigmentosa and which slowly progresses over decades. A second rapidly progressive form of mastocytosis presents with lymphadenopathy and peripheral eosinophilia.

Human bone marrow cultured in the presence of lectin-induced lymphocyte-derived growth factors yield increased numbers of mast cells/basophils after several weeks in culture. Human peripheral mononuclear cells produce factors which stimulate the division of mouse mast cells.

| | | | | | | | | | | | | | | | | |
|--|---|---------------------------------------|-----------|------------------------|------------------------------|-----------|---------|----------------------|----------------------|-----------|-----------------------|-------------------------|-----------|----------------------------------|-----------------|-----------|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00250-03 LCI | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders) Clinical and Basic Studies on Inflammatory Diseases of the Gastrointestinal Tract | | | | | | | | | | | | | | | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 45%;">Dean D. Metcalfe, M.D.</td> <td style="width: 20%;">Senior Clinical Investigator</td> <td style="width: 20%;">LCI/NIAID</td> </tr> <tr> <td rowspan="3">Others:</td> <td>Fred M. Atkins, M.D.</td> <td>Medical Staff Fellow</td> <td>LCI/NIAID</td> </tr> <tr> <td>Kim E. Barrett, Ph.D.</td> <td>Fogarty Visiting Fellow</td> <td>LCI/NIAID</td> </tr> <tr> <td>Ana Maria Saavedra-Delgado, M.D.</td> <td>Medical Officer</td> <td>LCI/NIAID</td> </tr> </table> | | | PI: | Dean D. Metcalfe, M.D. | Senior Clinical Investigator | LCI/NIAID | Others: | Fred M. Atkins, M.D. | Medical Staff Fellow | LCI/NIAID | Kim E. Barrett, Ph.D. | Fogarty Visiting Fellow | LCI/NIAID | Ana Maria Saavedra-Delgado, M.D. | Medical Officer | LCI/NIAID |
| PI: | Dean D. Metcalfe, M.D. | Senior Clinical Investigator | LCI/NIAID | | | | | | | | | | | | | |
| Others: | Fred M. Atkins, M.D. | Medical Staff Fellow | LCI/NIAID | | | | | | | | | | | | | |
| | Kim E. Barrett, Ph.D. | Fogarty Visiting Fellow | LCI/NIAID | | | | | | | | | | | | | |
| | Ana Maria Saavedra-Delgado, M.D. | Medical Officer | LCI/NIAID | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) None | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Clinical Investigation | | | | | | | | | | | | | | | | |
| SECTION Allergic Diseases Section | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | |
| TOTAL MAN-YEARS <div style="text-align: right;">1.80</div> | PROFESSIONAL: <div style="text-align: right;">1.80</div> | OTHER: | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.) <p>The degranulation of cultured mouse mucosal mast cells and rat peritoneal mast cells, but not human basophils, is inhibited by the drug sulphasalazine, used to treat inflammatory bowel disease. N-acetyl cysteine, given as a mucolytic agent in asthma and to treat acetomenophen overdose, has been shown to directly degranulate mast cells and basophils. This may help explain some adverse reactions to this drug.</p> <p>Mast cells, obtained from monkey gastrointestinal tissues, degranulate to immunologic stimuli and ionophores, but not to compound 48/80. Histamine release is inhibited by theophylline.</p> <p>Patients with a history of immediate adverse reactions to foods and whose symptoms are reproduced on challenge, are atopic, have multiple positive skin tests to foods and inhalants, have a positive skin test to the food in question, and by history are those with the most severe reactions. To date, approximately twenty-five patients with idiopathic anaphylaxis, and four with systemic mastocytosis have been challenged with sulfites. No clinical reactions have been observed, although plasma histamines elevated in three out of four patients with systemic mastocytosis.</p> <p>Patients receiving ATG had a rise in ATG-specific IgE.</p> | | | | | | | | | | | | | | | | |

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|--|---|--|-------------------------------|---------------------------------------|-----------|------------------------------|---------------------------------|-----------|--------------------|------------------------------------|-----------|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 A1 00269-03 LCI | | | | | | | | | |
| PERIOD COVERED October 1, 1983 through September 30, 1984 | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Phagocytic Cell Activation | | | | | | | | | | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Bruce E. Seligmann, Ph.D.</td> <td style="width: 33%;">Senior Staff Fellow</td> <td style="width: 33%;">LCI/NIAID</td> </tr> <tr> <td>Others: John I. Gallin, M.D.</td> <td>Chief, Bacterial Diseases</td> <td>LCI/NIAID</td> </tr> <tr> <td>Jean McKay</td> <td>Technician</td> <td>LCI/NIAID</td> </tr> </table> | | | PI: Bruce E. Seligmann, Ph.D. | Senior Staff Fellow | LCI/NIAID | Others: John I. Gallin, M.D. | Chief, Bacterial Diseases | LCI/NIAID | Jean McKay | Technician | LCI/NIAID |
| PI: Bruce E. Seligmann, Ph.D. | Senior Staff Fellow | LCI/NIAID | | | | | | | | | |
| Others: John I. Gallin, M.D. | Chief, Bacterial Diseases | LCI/NIAID | | | | | | | | | |
| Jean McKay | Technician | LCI/NIAID | | | | | | | | | |
| COOPERATING UNITS (if any) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">Marc M. Friedman, Ph.D.</td> <td style="width: 33%;">Dept. Microbiol. Georgetown Med. Ctr.</td> <td style="width: 33%;"></td> </tr> <tr> <td>Mark P. Fletcher, M.D.</td> <td>University of California, Davis</td> <td></td> </tr> <tr> <td>Harry Malech, M.D.</td> <td>Yale University School of Medicine</td> <td></td> </tr> </table> | | | Marc M. Friedman, Ph.D. | Dept. Microbiol. Georgetown Med. Ctr. | | Mark P. Fletcher, M.D. | University of California, Davis | | Harry Malech, M.D. | Yale University School of Medicine | |
| Marc M. Friedman, Ph.D. | Dept. Microbiol. Georgetown Med. Ctr. | | | | | | | | | | |
| Mark P. Fletcher, M.D. | University of California, Davis | | | | | | | | | | |
| Harry Malech, M.D. | Yale University School of Medicine | | | | | | | | | | |
| LAB/BRANCH Laboratory of Clinical Investigation | | | | | | | | | | | |
| SECTION Bacterial Diseases Section | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20205 | | | | | | | | | | | |
| TOTAL MAN-YEARS: <div style="text-align: center;">2</div> | PROFESSIONAL: <div style="text-align: center;">1</div> | OTHER: <div style="text-align: center;">1</div> | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | | | | | | | | | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>A number of <u>antibodies</u> were <u>screened</u> for binding to human peripheral neutrophils and one was identified (<u>31D8</u>) which binds heterogeneously, identifying two different populations of neutrophils. Using this antibody and flow cytometry we found that neutrophils which are 31D8-positive <u>express a unique receptor</u> for and respond to stimulation by the chemoattractant <u>f met-leu-phe</u> with membrane potential depolarization, generation of superoxide anion, and chemotaxis. 31D8-negative cells do not respond or exhibit chemotaxis, unless they are first <u>"primed" in vitro</u>. After <u>"priming"</u> these 31D8-negative cells express the same unique receptor as the 31D8-positive cells, though they do not express the 31D8 antigen. This evidence clearly demonstrates there are two types of neutrophils circulating in the peripheral blood of humans <u>with different functional capacity</u> which are likely to be modulated in vivo.</p> <p>In other studies we have investigated the <u>role of surface carbohydrate in neutrophil "priming"</u> using the lectin wheat germ agglutinin (WGA). <u>WGA stimulates neutrophils to depolarize, produce superoxide, hydrogen peroxide, and aggregate, as does f met-leu-phe</u>. In addition, <u>there is a specific synergy between WGA and f met-leu-phe</u> which results in a substantial increase superoxide/hydrogen peroxide production, not seen with other agents.</p> <p>In related studies <u>methods were developed to measure the oxidative burst and secretion of azurophil granule contents by neutrophils using flow cytometry</u>. This has enabled us to simultaneously measure either of these functions as well as two other parameters (such as binding of stimulating ligand, antibody binding, membrane potential response, or intracellular calcium levels).</p> | | | | | | | | | | | |

| | | |
|---|----------------------|---|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 A1 00270-03 LCI |
| PERIOD COVERED October 1, 1983 through September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders) Tubulin Tyrosinolation in Normal and Abnormal Human Neutrophils | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Jayasree Nath, Ph.D. Expert LCI/NIAID Other: John I. Gallin, M.D. Chief, Bacterial Diseases LCI/NIAID | | |
| COOPERATING UNITS (if any) Dr. Martin Flavin, Laboratory of Cell Biology, NHLBI, NIH | | |
| LAB/BRANCH Laboratory of Clinical Investigation | | |
| SECTION Bacterial Diseases Section | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20205 | | |
| TOTAL MAN-YEARS 1.1 | PROFESSIONAL: 1.1 | OTHER: |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) <p> The biochemical modification of human neutrophil (PMN) tubulin microtubules was studied by monitoring the post-translational incorporation of tyrosine into tubulin α-chains. The data indicate that a variety of stimuli, such as the peptide chemoattractant fmet-leu-phe, the Ca^{2+}-ionophore A23187 and phorbol myristate acetate (PMA), cause a 2-3 fold stimulation of PMN tubulin tyrosinolation that is closely associated with the PMN redox state. The above stimuli fail to induce stimulation in normal PMN under reduced and anaerobic conditions and also in PMN from patients with chronic granulomatous disease, which are deficient in oxidative metabolism. Extracellular Ca^{2+} and the cellular Ca^{2+}-regulatory protein, calmodulin, are necessary for the modulation of PMN tubulin tyrosinolation, although there are significant differences in the Ca^{2+}-sensitivity of various stimuli. In studies to localize the reaction at a sub-cellular level, tightly associated tubulin has been detected in isolated PMN fractions enriched in plasma membranes, azurophil granules and specific granules, that could be tyrosinolated in vitro in the presence of exogenous ligase, the enzyme that catalyzes the reaction. Endogenous ligase was detected in PMN azurophil granules. This is the first demonstration of an intracellular organelle-associated ligase in its functional form. Further studies utilizing a monoclonal antibody, YL 1/2 which recognizes only the tyrosinolated form of α-tubulin, reveal a preferential stimulation of tyrosinolation in plasma membrane-associated tubulin of fmet-leu-phe-stimulated PMN. A significant stimulation was also observed in the cytoplasmic tubulin fraction. Unlike in intact PMN, organelle-depleted PMN cytoplasts do not respond to fmet-leu-phe, although they have a significant basal level of tyrosinolation. These results provide further insights into the mechanism of modulation of tubulin tyrosinolation in PMN and demonstrate the sub-cellular localization of the reaction in resting and stimulated PMN. </p> | | |

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00271-03 LCI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Purification and Characterization of Serum Complement Proteins and Fragments

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---------|-------------------------|---------------------|-----------|
| PI: | Carl H. Hammer, Ph.D. | Senior Investigator | LCI/NIAID |
| Others: | Michael M. Frank, M.D. | Clinical Director | LCI/NIAID |
| | Louis Fries, M.D. | Med. Staff Fellow | LCI/NIAID |
| | Chaim Brickman, M.D. | Med. Staff Fellow | LCI/NIAID |
| | Lois Renfer | Research Biologist | LCI/NIAID |
| | Lawrence Prograis, M.D. | Med. Staff Fellow | LCI/NIAID |
| | Thomas Lawley, M.D. | Senior Investigator | DB/NCI |
| | Kim Yancey, M.D. | Med. Staff Fellow | DB/NCI |

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:

1.6

PROFESSIONAL:

1.6

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
 ☐ (b) Human tissues
 ☒ (c) Neither
- ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our previously reported methods for purification of complement components continue to be improved and updated according to our laboratory requirements and the properties of the isolated components (i.e., stability, concentration in plasma, and ease of isolation). Large scale isolation of C3, C4, C5, C9 and factor H has been well established and procedures for improved resolution of P, I, C2, B, C7, C8 and C6 early in their isolation have been developed, although the overall recovery of these latter components during isolation needs to be improved. We have adapted an affinity technique using C4b-Sepharose for the rapid, final purification with full recovery of human C2 from serum. C2 obtained this way is pure as judged by SDS-PAGE, alkaline PAGE and does not contain factor B, C7 or C4 by the immunoblotting technique. The only detectable contamination is a low level of IgA. Spontaneous loss of C3 activity associated with its isolation and storage requires more frequent purification of this labile component. The new isolation procedure we report was specifically designed to rapidly isolate C3 from smaller volumes of EDTA-plasma (60 ml) in three short steps. Pure C3 with full specific hemolytic activity can be prepared in three days with this protocol. Monospecific antibody to C1-In has been raised in a goat to this protein purified by our recently developed isolation scheme. The antibody to C1-In is being used to isolate a non-functional variant of C1-In from the plasma of a patient with an acquired form of hereditary angioedema. Methods to prepare C5a from citrated plasma have been finalized and the C5a obtained has been physico-chemically and biologically characterized. Final purification of C6 and C4 is in progress.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00272-03 LCI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Host Defense Against Pneumococcal Bacteremia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Eric Brown, M.D. Senior Investigator LCI/NIAID
Others: Richard Sveum, M.D. Medical Staff Fellow LCI/NIAID

COOPERATING UNITS (if any)

Thomas Chused, LMI, NIAID
Tsuneo Takahashi, American Red Cross

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland

TOTAL MAN-YEARS

1.75

PROFESSIONAL:

0.75

OTHER

1.0

CHECK APPROPRIATE BOX(ES)



(a) Human subjects



(b) Human tissues



(c) Neither



(a1) Minors



(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have demonstrated that neoantigens appear in human IgG after it has bound antigen. This was shown by raising antibodies in rabbits which reacted with antigen bound, but not monomeric or nonspecifically aggregated IgG. We have extended these studies to the development of monoclonal antibodies against human IgG in mice tolerized with monomeric or nonspecifically aggregated IgG. We have made several different monoclonal antibodies. Some recognize antigen bound IgG and plate bound monomeric IgG equally well. Some recognize monomeric IgG better than antigen bound IgG. We also have one antibody which recognizes antigen bound IgG better than monomeric plate bound IgG.

We have developed new fluorescent methods for the study of pneumococcal phagocytosis which allow us to separate the adherence phase and the ingestion phase of phagocytosis. Using this assay we have examined the nature of the receptors through which human monocytes and PMN adhere to bacteria and the receptors through which they ingest the adhered bacteria. We also have described rate constants for each of these reactions.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00273-03 LCI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Fibronectin in Opsonization and Phagocytosis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Eric Brown, M.D. Senior Investigator LCI/NIAID

Others: John Bohnsack, M.D. Medical Staff Fellow LCI/NIAID

COOPERATING UNITS (if any)

Tsuneo Takahashi, American Red Cross

George Martin and Hynda Kleinman, LDBA, NIDR

Erkki Ruoslahti and Michael Pierschbacher, La Jolla Cancer Research Foundation

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

1.5

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☒ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies have been extended on the role of fibronectin in phagocytosis by monocytes. Anti-fibronectin monoclonals have been used to purify fibronectin fragments. The ability of these fragments to 1) bind to monocytes and 2) enhance phagocytosis via the C3b receptor have been examined. We have shown that fibronectin fragments which bind well to monocytes are not sufficient to stimulate phagocytosis, and that another molecular fragment is required. We have localized that second fragment outside of a 70 kd fragment which contains the cell binding region of fibronectin. We have some evidence that this second signal comes from a region of the molecule around the gelatin binding domain and the heparin I domain.

We have initiated studies on the effects of laminin on monocyte phagocytosis. Mouse laminin increases ingestion of EC4b through a mechanism similar to the fibronectin effect. Anti laminin antibodies inhibit this phagocytosis which is dependent on laminin concentration and the amount of C4b on the opsonized erythrocytes. Laminin has some effect in the fluid phase, as well as when plated onto surfaces. The laminin receptor of cultured human monocytes is an approximately 80,000 dalton glycoprotein with less than 5,000 copies present on the cell surface.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00275-03 LCI

PERIOD COVERED

October 1, 1983 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Complement Receptor and C3 Mediated Opsonization

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---------|------------------------|--------------------|-----------|
| PI: | Thelma Gaither | Research Biologist | LCI/NIAID |
| Others: | Michael M. Frank, M.D. | Clinical Director | LCI/NIAID |
| | Kevin Proctor | Laboratory Worker | LCI/NIAID |
| | Irma Vargas | Biologist | LCI/NIAID |

COOPERATING UNITS, (if any)

Dr. John Gallin, LCI/NIAID
 Dr. Thomas Quinn, John Hopkins Medical School

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland

TOTAL MAN-YEARS

1.55

PROFESSIONAL:

1.05

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We examined the phagocytic activity of PMN from patients recovering from infection, using sheep erythrocytes opsonized with purified complement components and IgG as target cells. By controlling the input of IgG, the contribution of complement to phagocytic uptake could be assessed. By treating PMN with sodium azide, we were able to increase phagocytosis by several fold. We found that PMN from patients with chronic granulomatous disease (CGD) were similar to PMN of healthy controls in their ability to phagocytose particles coated with C3b and limiting inputs of IgG. However, when higher concentrations of IgG were used to sensitize cells and C3b was not present, CGD PMN were markedly more phagocytic than PMN from normal controls. This suggests that CGD, Fc receptor function is upregulated. PMN from other patients who were recovering from a variety of infections, were shown to have enhanced expression of C3b receptors. Our data suggests that there is enhanced ingestion of particles coated with C3b plus limited amounts of IgG in infected patients. Also particles bearing C1q, plus IgG showed enhanced ingestion in these patients, whereas strictly Fc mediated ingestion was similar to normal controls. Thus, infected patients show enhanced phagocytosis depending on how the target cells are opsonized. Unlike the control patients, the enhanced activity in CGD appears to be mediated by the Fc receptor.

Shinichi Inada, a visiting fellow, and others in our laboratory published that adhered monocytes bind particles coated with the C3b degradation fragment, C3d, under certain conditions of activation or differentiation. Now we have examined the complement receptor responsible for C3d binding and its physiologic function. We showed that C3d, like the fragments C3b and iC3b, enhances IgG mediated phagocytosis by monocytes. It appears that C3d binds to monocytes via the C3b and iC3b receptors which are apparently altered during the adherence process. These findings show for the first time that the C3d fragment has an opsonic function.

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| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER <div style="text-align: center; font-weight: bold;">Z01 AI 00276-03 LCI</div> |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies of the Membrane Attack Complex of Human Complement | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <div style="display: flex; justify-content: space-between;"> <div> PI: Keith Joiner, M.D. Others: Martin Sanders, M.D. Carl Hammer, Ph.D. Michael M. Frank, M.D. Mark Schmetz </div> <div> Senior Investigator Medical Staff Fellow Senior Investigator Clinical Director Med. Tech. </div> <div> LCI/NIAID LCI/NIAID LCI/NIAID LCI/NIAID </div> </div> | | |
| COOPERATING UNITS (if any) Dr. Moon Shin, University of Maryland, Baltimore, MD | | |
| LAB/BRANCH Laboratory of Clinical Investigation | | |
| SECTION Clinical Immunology Section | | |
| INSTITUTE AND LOCATION National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20205 | | |
| TOTAL MAN-YEARS: <div style="text-align: center; font-weight: bold;">1.5</div> | PROFESSIONAL: <div style="text-align: center; font-weight: bold;">1.4</div> | OTHER: <div style="text-align: center; font-weight: bold;">.1</div> |
| CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input checked="" type="checkbox"/> (b) Human tissues </div> <div> <input type="checkbox"/> (c) Neither </div> </div> | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Studies have continued on the characterization of monoclonal antibodies directed against complement component C9 and polyclonal Ab directed against neo-antigenic determinants of the membrane attack complex of complement C5b-9. These studies have demonstrated that only a small percentage of the Mab within ascites fluid was specific for C9, and this antibody was of low affinity. For these reasons, attempts are now underway to produce new clones recognizing C9. Studies with polyclonal antibody to neoantigens of the membrane attack complex have demonstrated low level but consistent cross-reactivity by ELISA of this antibody with an undefined constituent of serum. For these reasons polymerized C9 (poly C9) was prepared, and antibody directed against neoantigenic determinants within poly C9 was produced. This anti-poly C9 antibody has been thoroughly evaluated, and is known to react only with determinants on polymeric C9. Studies are underway with this antibody, looking for poly C9 deposition in the tissues and bloodstream of patients with various inflammatory and autoimmune disorders. The anti-poly C9 antibody is also being used to characterize poly C9 formation on bacteria and nucleated cells during the process of complement attack. | | |

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00277-0 3 LCI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanism of Serum Resistance in Bacteria

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|-----|-----------------------|---------------------|-----------|
| PI: | Keith A. Joiner, M.D. | Senior Investigator | LCI/NIAID |
|-----|-----------------------|---------------------|-----------|

| | | | |
|---------|------------------------|---------------------|-----------|
| Others: | Carl H. Hammer, Ph.D. | Senior Investigator | LCI/NIAID |
| | Michael M. Frank, M.D. | Clinical Director | LCI/NIAID |
| | Keith A. Warren | Med. Tech. | LCI/NIAID |
| | Mark A. Schmetz | Med. Tech. | LCI/NIAID |
| | Judith Falloon, M.D. | Med. Staff Fellow | LCI/NIAID |

COOPERATING UNITS (if any)

Nili Grossman and Loretta Leive, LCBG, NIADDK
 Robert Dourmashkin, St. Bartholemews Hospital, London, England

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

3.2

PROFESSIONAL

1.2

OTHER

2.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies have continued on the mechanism by which specific pathogenic bacteria evade killing by the serum complement system. Experiments with serum resistant *E. coli* and salmonella demonstrated that the C3 which was deposited on the bacterial surface by the alternative complement pathway bound predominantly to lipopolysaccharide (LPS) molecules through an ester linkage. Furthermore, C3 bound almost exclusively to the subset of LPS molecules bearing the longest O-polysaccharide (O-PS) side chains. Growth of salmonella under conditions which rendered the organism sensitive to serum killing resulted in attachment of C3 to molecules of LPS bearing shorter O-PS side chains. We concluded that LPS sterically hinders access of complement to otherwise susceptible sites on the outer membrane. Studies with 3 isogenic strains of *E. coli* varying in outer membrane composition and serum sensitivity showed that killing by complement was associated with deposition of C9 on the outer membrane in a form resistant to elution with salt or trypsin. Experiments have also continued on the mechanism of resistance to serum killing in *Neisseria gonorrhoeae* (GC). We demonstrated that bactericidal but not non-bactericidal C5b-9 was associated with distinctive outer membrane proteins when extracted from serum treated GC with detergent. Additionally, bactericidal and non-bactericidal C5b-9 had different associations with outer membrane constituents by electron microscopy. Finally, we found that monoclonal antibodies directed against identical or closely related epitopes on gonococcal protein I varied markedly in bactericidal activity, despite leading to equivalent complement deposition on the bacterial surface.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00278-03 LCI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of the Late Components of the Complement Cascade

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---------|------------------------|----------------------|-----------|
| PI: | Carl H. Hammer, Ph.D. | Senior Investigator | LCI/NIAID |
| Others: | Michael M. Frank, M.D. | Clinical Director | LCI/NIAID |
| | Lois Renfer | Research Biologist | LCI/NIAID |
| | Thomas Lawley, M.D. | Senior Investigator | DB/NCI |
| | Kim Yancey, M.D. | Medical Staff Fellow | DB/NCI |

COOPERATING UNITS (if any)

Dr. Hattie Gresham, Department of Medicine, University of Alabama

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:

1.1

PROFESSIONAL:

1.1

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Recent work has resulted in a simplified approach to the isolation of highly purified C5a from a purified C5 as well as citrated plasma. Specific antibody staining of nitrocellulose containing C5a electrophoretically transferred from SDS-PAGE gels confirms the presence, size and purity of this C5 cleavage fragment. Human C5a prepared by this method was shown to be a potent chemoattractant for neutrophils and monocytes, and an aggregator of neutrophils. C5a prepared from purified human C5 with zymosan bound alternate pathway convertase was used for in vivo skin test studies in normal volunteers. These studies revealed that C5a produced immediate wheal and flare reactions in all individuals, and was active in doses as low as 1 ng (10^{-13} mole). A clear dose-response effect was obtained between 1 and 120 ng. Comparison of in vivo reactivity of human C5a with C3a, histamine, 48/80, and morphine sulfate demonstrated that C5a was the most potent mediator of wheal and flare reactions. In addition several pharmacologic agents commonly employed to treat dermatologic and/or allergic disorders were tested for their ability to modulate cutaneous reactivity to C5a. This study is the first detailed analysis of the cutaneous reaction pattern to C5a in man and demonstrates that C5a is a potent mediator of inflammation in human skin in vivo.

C5a generation in vivo by cell bound classical pathway convertase (EAC1423) was examined in relation to the terminal complement components C6-9. It was shown that the presence of terminal components during activation of C5 allowed generation of significantly greater levels of C5a antigen and biological activity although consumption of C5 substrate was similar whether or not the terminal components were present. C5a antigen detected by radioimmunoassay correlated with C5a biological activity assessed by polymorphonuclear myeloperoxidase release and thus describes a new role for the human terminal complement components in generation of biologically active C5a.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00279-03 LCI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Studies on Mucous Glycoproteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

| | | | |
|---------|--------------------------|---------------------------------|-----------|
| PI: | Michael A. Kaliner, M.D. | Head, Allergic Diseases Section | LCI/NIAID |
| Others: | Zvi Marom, M.D. | Medical Staff Fellow | LCI/NIAID |
| | James Shelhamer, M.D. | Senior Investigator | CCM/CC |
| | Carolea Logun | Biologist | CCM/CC |

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Allergic Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

1.0

PROFESSIONAL

1.0

OTHER

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Mucus secretion is a normal function of respiratory mucous membranes. Models for measurement of mucus production by cultured human bronchial and nasal mucosae have been developed in order to examine the controls of mucus secretion. In addition to neurohormones and mediators of allergy, airways react to products generated by pulmonary macrophages and peripheral mononuclear cells with increased mucous glycoprotein secretion. The macrophage and mononuclear derived secretagogues are collectively being called macrophage/mononuclear cell derived mucus secretagogues (MMS). Both are generated by mononuclear cells phagocytosing zymosan or by surface activation with protein A-containing *S. aureus*. The generation of MMS is maximal after 4 hours, requires new protein synthesis by a cycloheximide-sensitive pathway and MMS is not stored intracellularly. MMS from mononuclear cells is small (2,000 daltons) and focuses with a PI of 5.1.

Activation of complement leads to anaphylatoxin generation. Current studies indicate that anaphylatoxins may be formed in pulmonary inflammatory processes. Therefore, the effects of human C3a upon mucus release were examined. C3a (and C5a) cause a dose-related stimulation of mucus secretion, maximal at 1-4 hours, apparently not requiring mast cell activation and not reproduced by C3a des arg. Thus, complement derived anaphylatoxins may also participate in mucus secretion.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00354-02 LCI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunoregulatory Defects in Inflammatory Bowel Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Stephen P. James, M.D., Senior Investigator, Mucosal Immunity Section, LCI/NIAID

Warren Strober, M.D., Chief, Mucosal Immunity Section, LCI, NIAID

COOPERATING UNITS (if any)

Claudio Fiocchi, M. D., Cleveland Clinic Foundation, Cleveland, Ohio

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mucosal Immunity Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD. 20205

TOTAL MAN-YEARS

2.3

PROFESSIONAL

1.3

OTHER

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☒ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this investigation was to define T lymphocyte phenotypes and immunoregulatory function in the intestine of patients with Crohn's disease. Crohn's disease and control patients undergoing surgical resection were studied. Intestinal lymphocytes were isolated by enzymatic digestion and compared to peripheral blood lymphocytes. T lymphocyte phenotypes were determined using monoclonal antibodies and flow cytometry. Helper and suppressor T cell function were studied by addition of patient lymphocytes to co-cultures containing normal lymphocytes and pokeweed mitogen. Supernatant immunoglobulin was measured by RIA.

The results of flow cytometry studies indicated that both Crohn's and control patients had a diminished proportion of Leu 3+ cells and a normal proportion of Leu 2+ lymphocytes in the intestine, compared to peripheral blood. Lymphocytes from both Crohn's and control patients were similar to autologous peripheral blood T lymphocytes in their capacity to provide help. Neither Crohn's nor control intestinal T lymphocytes suppressed immunoglobulin synthesis unless they were pre-activated with Con A or enriched for OKT8+ suppressor cells.

In summary, first, normal intestinal lymphocytes are heterogenous with regard to T cell phenotypes and helper and suppressor T cell function; the predominant immunoregulatory effect of normal intestinal lymphocytes is to provide help under the conditions employed. Secondly, in contrast to patients with mild or inactive Crohn's disease, intestinal and peripheral blood lymphocytes of patients with moderate to severe activity of Crohn's disease have T lymphocyte phenotypes and immunoregulatory function which are similar to that of control lymphocytes. These findings may indicate that active intestinal inflammation in Crohn's disease may in part be due to inadequate recruitment or activation of suppressor cells in the intestine.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00355-02 LCI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders)

Studies of Immunoregulatory Defects Present in Primary Biliary Cirrhosis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Stephen P. James, Senior Investigator, Mucosal Immunity Section, LCI, NIAID

COOPERATING UNITS (if any)

Anthony Jones, Liver Unit, NIAMDDK, NIH

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mucosal Immunity Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD. 20205

TOTAL MAN-YEARS

0

PROFESSIONAL

0

OTHER

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☒

(b) Human tissues

☐

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

This project was inactive during FY 1984, but has not been discontinued.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00356-02 LCI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of the Regulation of IgA Immunoqlobulin Synthesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Warren Strober, M.D., Chief, Mucosal Immunity Section, LCI/NIAID

COOPERATING UNITS (if any)

David Jacobs, M.D., Staff Associate, Mucosal Immunity Section, LCI/NIAID

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mucosal Immunity Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD. 20205

TOTAL MAN-YEARS:

2.3

PROFESSIONAL:

2.3

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to delineate the cellular mechanisms which explain the selective differentiation of IgA B cells in mucosal lymphoid follicles, Peyer's patches. Initially, we showed that B cells bearing surface IgA (sIgA B cells) arise from B cells bearing surface IgM (sIgM B cells) only when the latter are co-cultured with a particular kind of T cell derived from the Peyer's patch, so-called switch T cells. The latter were found in Con A-stimulated clonal T cell populations and required a polyclonal stimulant (LPS) to exert their effect. The switch T cells did not act on surface IgG-bearing T cells and did not cause sIgA B cells to undergo terminal differentiation into IgA-producing plasma cells. Thus the switch T cells were class-specific and appear to act at a fundamental level to influence the course of Ig-gene rearrangements.

One of the important unanswered questions concerning the switch T cell is whether it is an antigen-reactive cell that brings about switch at the same time it mediates more conventional helper T cell functions, or whether it is an auxiliary cell which is activated during the course of an antigen-driven immune response, but is not itself stimulated by antigen. In the present series of experiments we addressed this question by studying the capacity of switch T cells to proliferate in culture as a result of exposure to autologous and allogeneic cells of various sources. We found that cloned T cells obtained from Peyer's patches and having switching capacity, proliferated in culture when exposed to Con-A activated T cells, LPS-activated B cells and LPS-activated macrophages, but not to unstimulated T cells. This stimulation was blocked by addition to the culture of monoclonal antibodies against appropriate I-A antigens but not antibodies against inappropriate I-A antigens or antibodies against D/K antigens. Thus, the switch T cells do in fact proliferate when exposed to autologous I-A antigens and are thus autoreactive cells. These results make it very likely that switch T cells are auxiliary cells that are activated by autologous I-A antigens that are exposed during cell activation induced by antigen.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00357-02 LCI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of the Autologous Mixed Lymphocyte Reaction

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Warren Strober, M.D., Chief, Mucosal Immunity Section, LCI/NIAID

COOPERATING UNITS (if any)

Hiroyuki, Kotani, M. D., Visiting Associate, Mucosal Immunity Section, LCI/NIAID

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mucosal Immunity Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD. 20205

TOTAL MAN-YEARS

1.3

PROFESSIONAL

1.3

OTHER

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unabbreviated type. Do not exceed the space provided.)

This project is centered on the study of the autologous mixed lymphocyte reaction (AMLR), the proliferative response of T cells brought about by exposure to autologous B cells or macrophages. In the previous period we generated autoreactive T cell lines by repeatedly stimulating T cells with autologous non-T cells (B cells and macrophages) in the presence of IL-2. We showed that such lines respond to autologous but not allogeneic cells and, in addition, do not respond to mitogens and representative antigens. A key finding was that the lines were comprised of two sub-populations which bore the Leu 2 or the Leu 3 antigens; the former (Leu 2-positive) population mediated NK-like cytotoxicity, whereas the latter (Leu 3-positive) population were capable of killing autologous B cells. These studies thus raised the possibility that autoreactive cells may participate in autoimmune reactions.

In the present series of studies we determined the immunoregulatory activity of an autoreactive T cell lines and clones. In particular, we derived a cloned T cell line by the limiting dilution method from a continuously growing "bulk" cell line. The cloned cell was OKT4 positive but was nevertheless a suppressor T cell in that it suppressed Ig production by indicator cultures of fresh T cells (and B cells stimulated by pokeweed mitogen. The suppressor activity was exerted directly on B cells since it was observed in cultures of B cells stimulated by EB virus in the absence of T cells. The T4 suppressor cell line was a suppressor-effect population in that it suppressed in the absence of added OKT8-positive cells. Finally, the T4 suppressor cell line elaborated a factor which inhibits Ig synthesis at low concentrations. These studies indicate that suppressor cells are generated during the autologous MLR which are OKT4-positive. These regulatory cells address B cell function directly and may thus have a particular role in the pathogenesis of autoimmunity and in the B cell hyperreactivity observed in patients with AIDS.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00358-02 LCI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunopathogenesis of Chlamydia trachomatis Infection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Thomas C. Quinn, M.D., LCI, NIAID

COOPERATING UNITS (if any)

Johns Hopkins University School of Medicine: Mike Spence, Frank Polk, Beth Kappus
 University of California: Julius Schacter

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.6

PROFESSIONAL:

0.6

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Chlamydia trachomatis is the most commonly sexually transmitted pathogen in the United States and it is known to cause trachoma, conjunctivitis, pneumonia of the newborn and genital infections. The development of a monoclonal antibody for C. trachomatis has enabled us to screen large numbers of individuals for C. trachomatis by examining smears of genital secretions on slides stained with monoclonal antibody. A total of 1,080 patients were screened for C. trachomatis infection. The overall prevalence rate was 15.3% and the sensitivity and specificity of the direct monoclonal tests compared to culture was 88.7% and 98.5% respectively. Future aspects of this work will determine the sensitivity and specificity of this assay in detecting C. trachomatis infection in other physical sites and populations, such as newborn conjunctivitis in the U.S. and Africa, rectal infections in homosexual men, and low risk populations such as family planning clinics.

In order to further study the immunopathogenesis and efficacy of therapeutic regimens for C. trachomatis, we established acute fallopian tube infection (salpingitis) in 10 cynomolgus monkeys by intratubal inoculation with C. trachomatis. Similarly, we have also established rectal infections in primates with C. trachomatis which mimic granulomatous colitis in man both clinically and histopathologically. Intratubal and intrarectal cultures remain positive in these monkeys for C. trachomatis for six weeks post inoculation. Serial histologic examination of fallopian tube and rectal tissue demonstrate a mild acute polymorphonuclear response followed by mononuclear infiltration of the submucosa and mucosa by day 21 post inoculation. Reinoculation induces a prompt inflammatory response which consists primarily of polymorphonuclear cells and mononuclear cells. The presence of C. trachomatis was demonstrated in secretory cells by immunofluorescence and immunoperoxidase staining with monoclonal antibody to C. trachomatis. These animal studies provide a unique model to examine in detail the immunopathogenesis of chlamydial infection. Further studies will emphasize the effect of infection on mucosal and systemic immune responses.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00359-02 LCI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of ELISA Assays For Intestinal Protozoans

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Thomas C. Quinn, M.D., LCI/NIH

COOPERATING UNITS (if any)

Johns Hopkins University School of Medicine: Robert Yolken, Beth Ungar
 National Institute of Allergy and Infectious Diseases: Theodore Nash

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology

INSTITUTE AND LOCATION

NIH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

0.8

PROFESSIONAL

0.8

OTHER

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (a1) Minors☐ (a2) Interviews☐ (b) Human tissues☐ (c) Neither

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Intestinal infections with protozoan parasites such as Entamoeba histolytica, Giardia lamblia, Cryptosporidia, and Isospora belli are frequently found in homosexual men, individuals living in tropical areas, and travelers. Organisms such as Cryptosporidia and Isospora belli are being found high prevalence rates in tropical countries and in patients with the acquired immune deficiency syndrome (AIDS). Enzyme linked immunosorbent assays (ELISA) for the detection of pathogenic antigen in stool specimens offer the advantage of being rapid, easy to perform on large numbers of specimens, standardized without difficulty and adjunctive in epidemiologic studies. We have recently developed indirect double antibody ELISA systems to detect G. lamblia and E. histolytica in single stool specimens.

Homosexual men at risk for AIDS have recently been screened for these parasites with the above assays. For Giardia, stool specimens were positive by ELISA in 31 of 35 (88%) of patients with giardiasis by multiple stool exams; negative specimens came from patients with low number of parasites in the stool. In 10 patients followed prospectively, stools became negative by ELISA after successful treatment. Stool specimens were positive by ELISA in only 9 of 220 (4%) of patients without demonstrable giardia in their stool. For E. histolytica, 57 of 62 (92%) patients infected with E. histolytica were positive by ELISA. Five of 193 (3%) from patients without demonstrable E. histolytica in their stool were positive by ELISA. Coinfection with multiple other parasites did not appear to affect the sensitivity or specificity of these tests. An ELISA for the adequate detection for Cryptosporidia or Isospora has so far not been possible, utilizing a variety of different antibody assays. Rapid diagnosis of these pathogens has been accomplished by the auramine/acid fast stain. This stain has enabled us to detect these two pathogens in 4% and 1% respectively of the homosexual population which was screened. The significance of this project is to utilize rapid diagnostic tests for intestinal protozoans to detect infection and carriage of these protozoans in a high risk population for AIDS. Future aspects of this project will entail the screening of patients living in endemic areas such as Zaire and correlation with epidemiologic, immunologic and clinical features of the intestinal disease caused by these pathogens.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00360-02 LCI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Reticuloendothelial Function In Patients With AIDS

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Thomas C. Quinn, M.D. LCI/NIH

COOPERATING UNITS (if any)

Johns Hopkins University School of Medicine
 Laboratory of Clinical Investigation: b. Bender, M. Frank, Chain, brickman
 National Cancer Institute: T. Lawley

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology

INSTITUTE AND LOCATION

NIH, NIH Bethesda, Maryland 20205

TOTAL MAN-YEARS:

2.0

PROFESSIONAL:

1.4

OTHER:

0.6

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The acquired immune deficiency syndrome (AIDS) is characterized by infection with multiple opportunistic pathogens which are normally removed from circulation and killed by the phagocytic cells of the reticuloendothelial system (RES). Since these infections are readily disseminated in patients with AIDS, we prospectively analyzed in vivo RES function by measuring Fc receptor clearance rates of anti-Rho IgG antibody sensitized, ⁵¹Cr labelled autologous erythrocytes in 15 patients with AIDS, 9 with AIDS related illnesses, 5 healthy homosexual men, 7 healthy heterosexual men, and 5 patients with mycobacterial infection but without AIDS. In addition, we have also started analyses of the in vivo clearance rates of C3b-coated erythrocytes in order to examine complement receptor clearance rates. Eleven of 15 AIDS patients (p = 0.0005 vs controls) and 2 of 9 patients with AIDS related illness (p = NS vs controls) had prolonged Fc specific clearance rates when compared to healthy homosexual or heterosexual men. In contrast, patients with mycobacterial infections without AIDS had significantly more rapid clearance rates reflective of activated macrophage function. There was no association of clearance half times in AIDS patients with levels of circulating immune complexes, C3, C4, CH50, levels of T helper or T suppressor cells, or HLA phenotype. Likewise, C3b clearance rates were also markedly abnormal compared to healthy homosexual men and thus far there have been no correlation with any of the above immunologic parameters. However, defective clearance of both IgG and C3b coated red cells was correlated with prognosis: 6 of 11 patients with defective clearance rates died during the follow-up period as compared to 1 in 4 with normal clearance rates. One patient with AIDS who had a normal clearance rate had been receiving gamma interferon therapy. Data from this patient and from other in vitro studies suggests that gamma interferon may play an important role in altering macrophage activity in AIDS patients enabling them to resist disseminated opportunistic infections.

In summary, the significance of this project is to provide in vivo evidence in AIDS of immunologic dysfunction of the reticuloendothelial system, a target organ frequently effected by opportunistic pathogens, and to provide evidence for the potential use of immunomodulators in the treatment of AIDS.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00361-02 LCI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunologic Alterations In Patients With AIDS In The U.S. and Zaire

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Thomas C. Quinn, Senior Investigator, LCI/NIH

COOPERATING UNITS (if any) Johns Hopkins University School of Medicine: J. Sheridan

Centers for Disease Control: J. McCormick

National Institute of Allergy and Infectious Diseases: T. Folks, K. Sell

The Tropical Medicine Institute of Antwerp, Belgium: Peter Piot

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology

INSTITUTE AND LOCATION

NIH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

2.5

PROFESSIONAL

1.5

OTHER

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The common denominator of patients with the acquired immune deficiency syndrome (AIDS) appears to be a broad spectrum of cellular immune dysfunction. Previous work in our laboratory has clearly demonstrated that macrophages of the reticuloendothelial system are defective in vivo, and preliminary evidence demonstrates that peripheral monocytes are also functionally abnormal in terms of microbiocidal activity, chemotaxis and phagocytic activity. However, we believe that these macrophage and RES defects are secondary to a more primary defect of T-helper lymphocytes. We have demonstrated that lymphocytes from AIDS patients are unresponsive to nonspecific and viral specific antigens in their ability to develop a lymphocyte blastogenic response and in the production of lymphokines (leukocyte inhibition factor or macrophage inhibition factor). Addition of interleukin-1 to antigen stimulated cultures enhanced lymphokine production in heterosexual controls and homosexual lymphadenopathy groups, but not in AIDS patients. Addition of IL-2 did not modulate lymphokine production in the control or AIDS patients. These data support the finding that IL-1 or IL-2 may be ineffective in the treatment of AIDS patients late in the disease but may have some immunomodulating effect earlier in the disease process as in lymphadenopathy patients.

Work in this laboratory has now shifted focus onto the immunologic manifestations of AIDS in Zaire. Preliminary studies from our laboratory demonstrated an annual case rate in Zaire greater than any other site in the world (17/100,000). Immunologic features of these patients were similar to that identified in the United States, but immunologic abnormalities were also present in 25% of control patients with parasitic infections without AIDS. These included elevated circulating immune complexes, decreased T helper cells, hypergammaglobulinemia and decreased blastogenic responses. These preliminary data have prompted a more in-depth evaluation of the immunologic, clinical and epidemiologic features of AIDS in Zaire. Early data has confirmed the finding of a retrovirus (HTLV-III/LAV) in 95% of Zairian AIDS patients. The significance of this project is to further develop our understanding of the etiology and immunopathogenesis of AIDS in U.S. and in Zaire.

| | | |
|--|---|---|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER <div style="text-align: center; font-weight: bold;">Z01 AI 00379-02 LCI</div> |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies of DNA Viruses and Other Possible Agents in AIDS Patients | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) | | |
| PI: S.E. Straus, Senior Investigator, LCI, NIAID OTHER: H. Takiff (until 4/20/84) Medical Staff Associate LCI, NIAID J. Rooney Medical Staff Associate LCI, NIAID | | |
| COOPERATING UNITS (if any) G. Armstrong and G. Quinnan (BOB/FDA), H. Masur (CC, NIH), C. Lane and A.S. Fauci (LIR, NIAID). | | |
| LAB/BRANCH Laboratory of Clinical Investigation | | |
| SECTION Medical Virology Section | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MAN-YEARS: <div style="text-align: center; font-weight: bold;">0.5</div> | PROFESSIONAL: <div style="text-align: center; font-weight: bold;">0.25</div> | OTHER: <div style="text-align: center; font-weight: bold;">0.25</div> |
| CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input checked="" type="checkbox"/> (b) Human tissues </div> <div> <input type="checkbox"/> (c) Neither </div> </div> | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <div style="text-align: center; padding: 20px;"> <p>For the past two years this laboratory has been involved in extensive studies of herpesvirus infections in AIDS patients. We have developed techniques in this laboratory to assess AIDS patient specimens for presence of conventional and fastidious adenoviruses and parvoviruses.</p> </div> | | |

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00396-01 LCI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Complement Receptors: Regulation of Expression and Cell Biology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

| | | | |
|---------|----------------|--------------------------|-----------|
| PI: | J.J. O'Shea | Medical Staff Fellow | LCI/NIAID |
| Others: | E.J. Brown | Senior Investigator | LCI/NIAID |
| | B.E. Seligmann | | LCI/NIAID |
| | J.I. Gallin | Senior Investigator | LCI/NIAID |
| | T.J. Gaither | Biologist | LCI/NIAID |
| | M.M. Frank | Clinical Director, NIAID | LCI/NIAID |
| | T.M. Chused | Senior Investigator | LMI/NIAID |
| | M. Ochoa | Visiting Scientist | LCI/NIAID |

COOPERATING UNITS (if any)

M. Berger and Alan S. Cross
 Allergy Immunology Service and Department of Bacterial Diseases, Walter Reed Army Medical Center

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Allergic Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

1.25

PROFESSIONAL

1.25

OTHER

0.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

There are two distinct receptors identified for opsonic fragments of C3 on human phagocytic cells designated CR1 and CR3. Using monoclonal anti-receptor antibodies developed in this laboratory we demonstrated that both receptors rapidly augment their expression in response to a variety of biological agents including: the chemotactic peptides, fmet-leu-phe, C5a; phorbol myristate acetate; leukotriene B₄; a Raji cell derived lymphokine; and the calcium ionophore, A23187. We have further shown that this process is dependent upon extracellular calcium, calmodulin and a functional microtubule system. De novo protein synthesis is not required.

We further showed that the intracellular pool of CR3 cosediments with specific granules when disrupted neutrophils are fractionated on sucrose gradients. Moreover, organelle depleted neutrophil cytoplasm as well as neutrophils obtained from a patient lacking specific granules are unable to upmodulate receptor expression.

The importance of these studies is underscored by the description of a new clinical syndrome marked by recurrent infections. These individuals lack CR3 and their cells exhibit a variety of functional defects. In addition it has been recently shown that patients with systemic lupus erythematosus have a decreased expression of CR1 on their erythrocytes. Studies are underway to more fully understand the function of these receptors in health and disease.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00397-01 LCI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Interactions of C3b with Immunoglobulin G- Regulation of C3b Function By Antibody

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Louis F. Fries, III, M.D.

Medical Staff Fellow

LCI/NIAID

COOPERATING UNITS (if any)

none

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:

0.75

PROFESSIONAL:

0.75

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

IgG antibodies are excellent acceptors of nascent C3b and form covalent complexes with C3b in immune complexes and on bacterial surfaces. In addition, complement activation in serum leads to the generation of non-specific C3b-IgG complexes from bystander monomeric IgG. We have reported that C3b-IgG complexes can be readily formed in vitro and have delineated a purification schema which allows their preparation in good yield from purified C3 and IgG. C3b in such hetero-dimeric complexes is bound primarily to IgG heavy chains by an ester linkage. Such C3b shows a retarded rate of inactivation by factors H and I relative to free, uncomplexed C3b. This retardation of inactivation is primarily due to reduced affinity of C3b complexed to IgG for factor H. In the presence of adequate levels of factor H, the action of factor I on C3b-IgG heterodimers is not impeded by the IgG molecule. Substitution of an immunologically inert glycoprotein molecule, ceruloplasmin, for IgG results in hetero-dimeric complexes of C3b-ceruloplasmin. C3b in such complexes displays no retardation of inactivation by factor H and I, suggesting that inhibition of such inactivation may be a peculiar property of IgG. C3b-IgG complexes are much more efficient than C3b on a molecule for molecule basis in promoting alternative pathway complement activation. These data may explain the role of IgG in enhancing alternative pathway activity and also may have important bearing on our understanding of immune complex metabolism.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00398-01 LCI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Studies on the Interaction of Complement and Parasites

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

| | | | |
|---------|---------------|---|-----------|
| PI: | Keith Joiner | Senior Investigator | LCI/NIAID |
| Others: | Alan Sher | Chief, Immunology & Cell Biology Section | LPD/NIAID |
| | Sarah Heiny | Medical Technician | LPD/NIAID |
| | L.V. Kirchoff | Medical Staff Fellow | LPD/NIAID |

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland

TOTAL MAN-YEARS

.4

PROFESSIONAL

.3

OTHER

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
☐ (a1) Minors
☐ (a2) Interviews
- ☒ (b) Human tissues
- ☐ (c) Neither

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

We determined the membrane acceptor site for C3b deposited on epimastigotes (Epi) of *Trypanosoma cruzi* by the alternative pathway (AP). Epi (Tulahuen strain) were surface-iodinated with Iodogen, then incubated in 50% C8 deficient serum. Washed parasites were solubilized in detergent, and the lysate was applied to an anti-C3 column (A:C3). 15.6±1.6% of applied ¹²⁵I Epi protein bound specifically to A:C3 with .3% binding in controls. Bound ¹²⁵I Epi protein was eluted from A:C3 and analyzed by SDS-PAGE autoradiography. Non-serum incubated Epi showed 9 major iodinated bands ranging from 20 kd to 208 kd. Samples eluted from A:C3 with SDS showed a single diffuse band at 250-300kd, suggesting covalent attachment of C3b to ¹²⁵I Epi protein. Samples eluted from A:C3 with NH₂OH revealed a single major band at 72kd, suggesting that C3b attached almost exclusively to the 72 kd glycoprotein by a NH₂OH susceptible ester bond. An antiserum was prepared from lysates of serum treated Epi affinity purified on A:C3. This antiserum immunoprecipitated a single component of 72kd from surface iodinated Epi. These results are the first to evaluate the acceptor site for C3 deposition on a parasite, and show that C3b deposited by the AP binds preferentially to the 72kd glycoprotein, the major developmentally regulated surface antigen of Epi.

LABORATORY OF IMMUNOGENETICS
1984 Annual Report
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Annual Report
Laboratory of Immunogenetics
National Institute of Allergy and Infectious Diseases
October 1, 1983 to September 30, 1984

RESEARCH PROGRESS

The Laboratory of Immunogenetics investigates the multigene families that are involved in control of immune function. Our research emphasizes the structure and function of the genes and their products as well as mechanisms for gene regulation. Recent investigations have utilized the major histocompatibility complex (MHC) of the human, rabbit and mouse and the immunoglobulin gene complexes of these species. A wide range of techniques at the molecular, serologic and functional level are used in these investigations.

STUDIES OF THE HUMAN MHC

Several experimental systems are being utilized to investigate the MHC complex of the human. These include direct molecular analyses of gene structure within this complex, genetic mapping studies using families with recombinant members, structural studies of variant HLA antigens and control of expression of genes within multigene families.

Human class II genes. A nucleotide sequence has been determined for cDNA clones and for a genomic DNA clone encoding the beta chain of the SB antigen (Long). With this primary structural information, it was possible for the first time to determine the evolutionary relationship of SB beta chains to other beta chains encoded within the HLA-D region of the human and to those of the murine H-2I region. These data indicate that the beta chain genes of DR (I-E equivalent), DC (I-A equivalent), and SB (no murine equivalent) antigens have diverged from one another to approximately the same extent. In order to further analyze the multiple nonallelic HLA class II genes a library of cDNA clones has been constructed from mRNA of an HLA homozygous mutant B cell line. These genes will be used in transfection experiments aimed at dissection of the respective roles of the various HLA-D gene products in alloreactions and in antigen presentation to T cells (Tonnelie).

Gene order within the human MHC. DNA restriction enzyme polymorphisms within the HLA complex were studied in eight families found to include individuals inheriting recombinant HLA haplotypes (Robinson). Cloned DNA corresponding to class I, class II and class III MHC genes were used as probes in Southern blot analyses. Polymorphisms in the size of the restriction fragment obtained from the various MHC genes were observed and these could be assigned to haplotypes within families. Results to date have made it possible to identify a previously undetected crossover within the SB region of one family and to localize genes encoding a complement component, C4, within the HLA complex between HLA-B and HLA-DR. In addition, these data have allowed us to distinguish between HLA-DR, DC and SB haplotypes that type identically and to define the location of crossover events in each recombinant haplotype. The availability of B cell lines from these completely typed family members will allow assignment of new polymorphisms as the human MHC is further explored.

Structural studies of human class II antigens. Studies using monoclonal antibody 33.1 have identified a class II antigen that is distinct in distribution on cells and organs from HLA-DR and that has characteristics of the products of the I-A locus in the mouse which is termed DC in the human (Li, Sogn). The product identified by monoclonal antibody 33.1 was compared to that identified by other DC specific antibodies and it was shown that 33.1 is identical to the antibody designated Leu 10. Another monoclonal antibody, Genox 3.53, recognizes a distinct determinant that may be present on the subset of 33.1 positive molecules, raising the question of molecular heterogeneity within the DC region. Alternatively, the differences between these similar antibodies may also reflect post synthetic modifications or artifacts of isolation. The availability of 33.1 and other antibodies that recognize products of the DR and SB loci allows use of these monoclonals to study various means to induce expression of the HLA-D region products. At present the enhancement of class II gene transcription upon treatment known to activate B cells is being examined (Kulaga). Human B cells in various states of activation are being examined by Northern blotting techniques in order to determine the degree of transcription of each individual class II MHC gene and to correlate specific enhancement of transcription with specific signals to the B cells.

Variants of human HLA-A3. It was previously observed that the human class I molecule HLA-A3 exists in several forms which although serologically indistinguishable can affect the recognition of HLA-A3 influenza specific cytotoxic T cells (CTL). Structural analyses have revealed that there are only minor differences among the molecules that are perceived as different by the CTL (van Schravendijk). Furthermore, the region of the molecule which is important for CTL recognition, appears to be not particularly important for recognition for serologic reagents as these changes are not observed upon serologic typing. Differences among the variants of HLA-A3 are being investigated by cloning of A3 specific genes and determination of their structure (Cowan). The cloned genes will be further used in transfection systems in order to test the CTL results and to insure that the differences are related solely to the HLA-A3 molecule and not to other products.

Studies on molecules encoded by infectious agents recognized by the immune system are being carried out in parallel with studies on the human MHC system (Coligan). Molecules other than those encoded by the MHC including the human T cell specific molecules, T3 and T8, are being investigated as are the products of human leukemia T cell viruses that appear to raise antibodies in exposed humans. In a related study, the antigenic variation in Herpes simplex virus type I glycoproteins is being investigated. It is the goal of these studies to understand at the molecule level the interactions between cells of the human immune system and common human viral pathogens.

IMMUNOGENETIC STUDIES IN ANIMAL MODELS

In addition to studies concerning the human MHC and its products several animal models are being investigated with specific questions concerning histocompatibility antigens and their expression. These systems include the murine system, the rabbit, and more recently, the hamster.

Murine MHC antigens. Studies continue concerning the primary structure of class I antigens expressed by the mouse. These species has approximately

36 class I genes per haploid genome and various numbers of these may be expressed by normal lymphoid cells. In the b haplotype only a single K-region and D-region product has been found. Recent studies for mice of the g haplotype have shown that there are at least three D-region molecules D^g , L^g and R^g expressed by animals with this haplotype (Lillehoj). The molecule encoded by R^g is readily distinguishable from those of D^g and L^g whereas D^g and L^g possess greater than 99% sequence homology. This indicates that they are products of very recently duplicated genes. Structural studies on the H-2K^k molecule have allowed extensive characterization of this molecule and will permit comparison of the H-2K^k to other H-2 molecules and further to examine mechanisms purposed for alternative splicing mechanisms of K-region antigens.

In addition to the antigens of the lymphoid tissue which are membrane bound, there is a soluble H-2 class I product produced in the liver and found solely in the liver and in the serum (Maloy). This molecule appears to have limited polymorphism and has been found in all strains so far tested. Because the molecule is soluble, certain problems encountered in working with membrane bound antigens are circumvented and attempts will be made to obtain sufficiently large quantities of this soluble molecule for x-ray crystallography and functional studies (Lew).

Studies of murine class II antigens in the d haplotype have concentrated on the question whether chains from the I-A and I-E subregions can intercombine. Products that are possibly comprised of chains from both regions have been identified and further structural studies will be required to prove the origin of their component chains.

Rabbit MHC genes and antigens. Studies of genes encoding MHC (RLA) antigens have indicated that only a single major transplantation antigen is expressed in homozygous animals. The RLA gene complex has been subjected to a detailed analysis at the molecular level in order to examine this difference from other MHC systems in which three or more class I antigens are normally expressed. A cDNA library was constructed from a T cell line, RL-5, derived from an inbred rabbit strain. cDNA clones corresponding to four unique transcripts were identified (Marche). The clone (pR9) corresponding to the single expressed antigen was represented 26 times among the 31 clones characterized. Two of the cDNA clones have been fully sequenced and analysis of three others is now in progress. A genomic clone corresponding to the expressed product has been identified and its sequence determined. These studies have revealed a striking similarity between human and rabbit class I genes both in the coding region sequences and in the intron/exon structure. Comparison of rabbit and human class I genes to those of the mouse, on the other hand, reveal large differences both in exon/intron length and in sequences found in the 3' coding and noncoding regions. Studies are under way to prepare antibodies directed against the expressed rabbit class I antigens and to prepare synthetic oligonucleotide probes in order to determine tissue expression of the various members of the rabbit class I family (Kindt).

Initial studies have been carried using class II gene probes for both alpha and beta chains of the SB, DC and DR subregions (Robinson). These studies have indicated that the rabbit is similar in complexity in the D-region to the human. That is to say, more complex than is the mouse. Further studies will emphasize the rabbit genes analogous to the SB region of

the human for comparison of structure and function analogous to the human system.

Rabbit T cell proteins. Previous investigations allowed characterization of a number of T cell specific glycoproteins present on the surface of rabbit lymphoid cells. These antibodies were used to delineate subpopulations of rabbit lymphocytes and in some cases the antigens have been characterized. Recent cell cloning experiments have allowed isolation of a population of rabbit T lymphocytes that bear receptors for the streptococcal carbohydrate antigen (Jackson). These cloned lines proliferate in response to addition of the bacterial vaccine or to the isolated soluble carbohydrate. Although the experiments were made difficult by the requirement for autologous presenter cells, it was possible to characterize a number of T cell clones and to determine that they were helper T cells.

More recent studies have utilized cDNA probes for the murine T cell antigen receptor to probe DNA from rabbit liver and from a rabbit T cell line. The T cell line was found to contain rearranged genes for the T cell receptor and this probe will now be used to screen cDNA libraries in order to obtain the cDNA corresponding to the transcript of this rearranged gene.

Studies of hamster proteins. A major effort has been made to clone the gene encoding hamster female protein (FP). This protein is a hormonally regulated component of the acute phase response. Messenger RNA from the liver of male and female hamsters has been prepared and translated *in vitro* (Sogn). The translation has been shown to contain material precipitable with antiserum to FP and it has been shown that there is more of this material in translated mRNA from females than from males. In addition, protein sequencing from a site of CNBr cleavage toward the carboxyl terminus of FP has identified a region from which the nucleotide coding sequence can be inferred with a low level of ambiguity. This should make possible the preparation of a synthetic nucleotide probe specific for FP.

IMMUNOGLOBULIN GENE EXPRESSION

The regulation of expression of genes in the rabbit κ immunoglobulin (Ig) locus is being investigated (Max). Domestic rabbits harbor Ig κ sequences that give rise to two intense bands when genomic Southern blots are probed with a fragment of the cloned κ lb4 gene. A κ 2b4 gene and a κ lb5 gene have been cloned; accumulated evidence derived from all three genes identifies the two intensely hybridizing Southern blot bands as nominal expressed κ isotype gene and a κ 2 isotype gene (Esworthy). The κ 2 gene is apparently not expressed in domestic rabbits, although there are no obvious defects in the coding sequences of the cloned κ 2b4 gene. The possibility that differential κ 1 vs κ 2 expression results from different regulatory sequences in the J κ -C κ intron is currently being explored by inserting these intron sequences into expression vectors to test their effects as enhancers of κ gene transcription. In an attempt to understand latent κ allotype expression in rabbits, we examined Southern blots of DNA from a rabbit in our colony that expressed latent allotype were examined; no "extra" Southern blot bands that might have encoded latent allotype were observed. The V κ gene family is also under investigation (Lieberman).

Studies of gene activation in human B cell development have shifted emphasis from the attempts to advance developmental stages of B cell lines in vitro to studies of the chromosomal state of regulatory regions, especially in the human κ gene. The high-resolution Southern blot technology developed for genomic DNA sequencing will be used to examine DNAase hypersensitivity sites in the human κ enhancer and to look for differences between κ -expressing and non-expressing cell lines with respect to DMS protection (footprinting) and Exonuclease III susceptibility in this region.

Annual Report
Laboratory of Immunogenetics
National Institute of Allergy and Infectious Diseases
October 1, 1983 to September 30, 1984

ADMINISTRATIVE REPORT

During the period covered by this report there has been turnover of several positions at the postdoctoral and expert level within the Laboratory of Immunogenetics. In addition, the Immunobiology Section of the laboratory, headed by Dr. Kenneth Sell, has been transferred to the Office of the Scientific Director as was the Peptide Synthesis laboratory of Dr. W. Lee Maloy. Dr. Maloy will continue to occupy laboratory space within the Laboratory of Immunogenetics whereas the Immunobiology Section has completely moved to space on the 11th floor of the ACRF.

The new laboratory program initiated by Dr. Eric Long was joined by Dr. Cecile Tonnelle from the CNRS-INSERM Institut in Marseille and by Dr. Rafick Sekaly from the Ludwig Institut in Lausanne. In the Immunogenetics Research Section, Dr. Susan Jackson left the laboratory to become an Assistant Professor of Microbiology at the University of Alabama in Birmingham and Dr. Xuan-mao Li has left to continue his training at Harvard University. Dr. Henrietta Kulaga from Georgetown University has recently joined this section to work with Dr. John Sogn and Dr. Marie-Christine Rebiere from the Institut Pasteur in Paris has joined the laboratory to work with Dr. Kindt. Dr. Joyce Schroer, Expert, has resigned and Dr. Christine LeGuern, currently Professor at University of Paris and Staff Member at the Institut Pasteur will take a similar position within the laboratory. Dr. Annie LeGuern will join the laboratory in the Immunogenetics Research Section. In the Membrane Antigen Section, Dr. Ana-Maria Diaz Primerano left to assume a faculty position at the University of Puerto Rico and Dr. Marie Rose van Schravendijk will return to a research position at Oxford University. Dr. Andrew Lew from the London School of Hygiene and Tropical Medicine, has joined Dr. Coligan's group during this period. Dr. Jeff Gimble has joined Dr. Max's group as a Medical Staff Fellow.

Annual Report
Laboratory of Immunogenetics
National Institute of Allergy and Infectious Diseases
October 1, 1983 to September 30, 1984

HONORS AND AWARDS

Dr. Kindt has presented invited lectures at the University of Paris, at the Institut Pasteur, at the Hopital Necker, and at the Ludwig Institut in Lausanne. He was invited as a discussant at an EMBO Workshop on idiotypic networks in Florence, Italy. He has resumed his position as Section Editor for molecular biology and molecular genetics for the Journal of Immunology and has been invited to join the Editorial Board of the Journal of Biological Chemistry. Dr. Kindt has also been named as a member of the Scientific Advisory Council of the American Cancer Society and has served as reviewer for program projects for the National Cancer Institute. Dr. Kindt has been invited to contribute editorials to the Scandinavian Journal of Immunology and to serve as a Guest Editor for an exchange of ideas in this journal. He continues to serve as Adjunct Professor of Immunology at Georgetown University. Dr. Edward Max has presented data from his laboratory in invited lectures at the Scripps Institute in La Jolla and at Harvard University. In addition, he has been invited to contribute an editorial to Nature concerning a theory based on his recent observations. Dr. John Sogn has participated as a teacher in a nationwide series of courses on hybridoma technology. Dr. Eric Long was a plenary lecturer at the 9th meeting of the American Association for Clinical Histocompatibility Testing and in addition presented a paper at the Third Annual H-2 HLA Cloning meeting in Strasbourg, France. Dr. Coligan was an invited speaker at Johns Hopkins University in a minisymposium on the aspects of the major histocompatibility complexes and has delivered invited lectures concerning his laboratory's work at the Dana-Farber Cancer Center, Albert Einstein College of Medicine, Roswell Park Memorial Institute and at the NIADDK. Dr. Mary Ann Robinson has been invited to participate in a meeting on molecular techniques in HLA and to submit a paper concerning her recent work in this area. In addition, Dr. Robinson has been invited to serve as a consultant for Contract Review Board in NIAID and NCI.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00166-07 LIG

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders)

Characterization of Rabbit MHC Antigens

PRINCIPAL INVESTIGATOR (If not other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|--------|--------------------|-----------------|-----------|
| PI: | Thomas J. Kindt | Chief | LIG/NIAID |
| OTHER: | Patrice N. Marche | Visiting Fellow | LIG/NIAID |
| | Christian Le Guern | Expert | LIG/NIAID |

COOPERATING UNITS (if any)

LABORATORY

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

4.0

PROFESSIONAL:

2.0

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Studies of genes encoding rabbit MHC (RLA) antigens have indicated that only a single major transplantation antigen is expressed in homozygous animals. The RLA gene complex has been subjected to a detailed analysis at the molecular level in order to exam this difference from other MHC systems in which three or more class I antigens are normally expressed. A cDNA library was constructed from a T cell line, RL-5, derived from an inbred rabbit strain. cDNA clones corresponding to four unique transcripts were identified. The clone (pR9) corresponding to the single expressed antigen was represented 26 times among the 31 clones characterized. Two of the cDNA clones have been fully sequenced and analysis of three others is now in progress. A genomic clone corresponding to the expressed product has been identified and its sequence determined. These studies have revealed a striking similarity between human and rabbit class I genes both in the coding region sequences and in the intron/exon structure. Comparison of rabbit and human class I genes to those of the mouse, on the other hand, reveal large differences both in exon/intron length and in sequences found in the 3' coding and noncoding regions. Studies are under way to prepare antibodies directed against the expressed rabbit class I antigens and to prepare synthetic oligonucleotide probes in order to determine tissue expression of the various members of the rabbit class I family.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00168-07 LIG

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Immunoglobulin Allotypes of Heavy and Light ChainsPRINCIPAL INVESTIGATOR (If not other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
Thomas J. Kindt

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetic Research Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

0

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Inactive during year.

| | | | | | | | | | | | | | | | | | |
|---|-----------------------------|--|-------------------------------|-----------------|-----------|---------------|--------------|-----------|--------------|--------------|-----------|------------|-----------------|-----------|--------------|--------|-----------|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00169-07 LIG | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders). Analysis of Murine and Human Transplantation Antigens and Genes | | | | | | | | | | | | | | | | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) PI: John E. Coligan Senior Investigator LIG/NIAID | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">OTHER: M. R. van Schravendijk</td> <td style="width: 30%;">Visiting Fellow</td> <td style="width: 30%;">LIG/NIAID</td> </tr> <tr> <td>Erik Lillehoj</td> <td>Staff Fellow</td> <td>LIG/NIAID</td> </tr> <tr> <td>Elliot Cowan</td> <td>Staff Fellow</td> <td>LIG/NIAID</td> </tr> <tr> <td>Andrew Lew</td> <td>Visiting Fellow</td> <td>LIG/NIAID</td> </tr> <tr> <td>W. Lee Maloy</td> <td>Expert</td> <td>LIG/NIAID</td> </tr> </table> | | | OTHER: M. R. van Schravendijk | Visiting Fellow | LIG/NIAID | Erik Lillehoj | Staff Fellow | LIG/NIAID | Elliot Cowan | Staff Fellow | LIG/NIAID | Andrew Lew | Visiting Fellow | LIG/NIAID | W. Lee Maloy | Expert | LIG/NIAID |
| OTHER: M. R. van Schravendijk | Visiting Fellow | LIG/NIAID | | | | | | | | | | | | | | | |
| Erik Lillehoj | Staff Fellow | LIG/NIAID | | | | | | | | | | | | | | | |
| Elliot Cowan | Staff Fellow | LIG/NIAID | | | | | | | | | | | | | | | |
| Andrew Lew | Visiting Fellow | LIG/NIAID | | | | | | | | | | | | | | | |
| W. Lee Maloy | Expert | LIG/NIAID | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) D. Sachs, NCI; W. Biddison NINCDS; T. Hansen, Washington University at St. Louis; John Freed, National Jewish Hospital; G. Jay, NCI. | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Immunogenetics | | | | | | | | | | | | | | | | | |
| SECTION Membrane Antigen Structure | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | |
| TOTAL MAN-YEARS: 4.2 | PROFESSIONAL: 2.7 | OTHER: 1.5 | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Human and murine MHC class I molecules which are integrally involved in the recognition of virally infected cells by cytotoxic T lymphocytes (CTL), are isolated and their primary structure analyzed. The goal of these studies is to gain an understanding in molecular terms of their function and antigenic properties as well as to gain knowledge of their evolutionary relationships. The H-2^q haplotype has been shown to encode 3 D-region molecules, D^q, L^q and R^q. R^q is readily distinguishable from D^q and L^q whereas D^q and L^q possess greater than 99% sequence homology suggesting that they are recently duplicated genes. Extensive structural analysis of the H-2K^k molecule isolated both by radiochemical and preparative methods was obtained. These data permitted comparison of the H-2K^k to other H-2 molecules and will prove useful for the study of H-2K^k mutant molecules. Using anti-peptide sera, the synthesis of a soluble liver-specific H-2 molecule has been demonstrated in all strains and the function of this molecule is being investigated. In the case of human class I molecules, HLA-A3 variants which affect the recognition of HLA-A3-influenza specific cytotoxic T cells have been identified. Structural analysis has revealed that the region of the molecule correlated with alterations in recognition is discrete involving changes in only several amino acid residues. This region of the molecule which is important for CTL recognition appears not to be particularly important for recognition by serological reagents.</p> | | | | | | | | | | | | | | | | | |

| | | |
|---|----------------------|---------------------------------------|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00170-07 LIG |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders) Studies of Human Histocompatibility Antigens | | |
| PRINCIPAL INVESTIGATOR (If not other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Eric O. Long Visiting Scientist LIG/NIAID | | |
| OTHER: Cecile Tonnelle Visiting Fellow LIG/NIAID Rafick Sekaly Visiting Fellow LIG/NIAID | | |
| COOPERATING UNITS (if any) David Eckels, Robert Hartzman, A. H. Johnson, Georgetown University. | | |
| LAB/BRANCH Laboratory of Immunogenetics | | |
| SECTION Immunogenetics Research Section | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MAN-YEARS: 2.4 | PROFESSIONAL: 1.4 | OTHER: 1 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The structure and the expression of the genes for human class II antigens of the major histocompatibility complex have been studied at the molecular level. The nucleotide sequence determined for a cDNA clone (derived from mRNA) and for part of a genomic DNA clone (derived from cellular DNA), both encoding the beta chain of an SB antigen, has defined the primary structure of an SB beta chain. It was possible for the first time to determine the evolutionary relationship of SB beta chains to other beta chains in the human HLA-D region and in the murine H-2 I-region. The beta chain genes of DR (I-E equivalent), DC (I-A equivalent) and SB (no murine equivalent) antigens have diverged from each other to approximately the same extent. In order to analyze further the multiple nonallelic HLA class II genes a library of cDNA clones has been constructed from mRNA of an HLA-hemizygous mutant B cell line. Several vectors have been constructed that will permit the expression of inserted cDNA sequences after transfection into eukaryotic cells. The availability of multiple human class II genes which can be expressed will lead to a dissection of their respective role in alloreactions and antigen presentation to T cells. </p> | | |

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00171-07 LIG

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic Studies on Rabbit Immunoglobulins and Other Serum Proteins

PRINCIPAL INVESTIGATOR (If not other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: John A. Sogn Research Chemist LIG/NIAID

OTHER: Henrietta Kulaga Staff Fellow LIG/NIAID
Susan Jackson Staff Fellow LIG/NIAID

COOPERATING UNITS (if any)

J. Coe, RML, NIAID and M. Temple, Georgetown University.

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

2.1

PROFESSIONAL:

1.4

OTHER:

0.7

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

A major effort has been made to clone the gene encoding hamster female protein (FP). This protein is a hormonally regulated component of the acute phase response. Messenger RNA from the liver of male and female hamsters has been prepared and translated in vitro. The translation has been shown to contain material precipitable with antiserum to FP and it has been shown that there is more of this material in translated mRNA from females than from males. In addition, protein sequencing from a site of CNBr cleavage toward the carboxyl terminus of FP has identified a region from which the nucleotide coding sequence can be inferred with a low level of ambiguity. This should make possible the preparation of a synthetic nucleotide probe specific for FP. In the course of work on FP, it has been of interest to investigate the class I major histocompatibility antigens of the hamster. Using a class I MHC probe from the rabbit it has been shown that the hamster has a large number of genes with very high homology to class I genes of other species. This eliminates one possible explanation for the low level of polymorphism observed in class I genes in this species.

During the year, the specificity of a panel of antibodies defining rabbit lymphocyte cell surface antigens has been further investigated. The possibility that some of these antibodies recognize glycolipids is being examined. Rabbit mouse hybridomas (RMH) which have been prepared in this laboratory are under study using the technique of in situ hybridization in an attempt to determine the site of integration of the rabbit immunoglobulin genes in the mouse genome.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00173-07 LIG

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders)

Control of Gene Expression

PRINCIPAL INVESTIGATOR (If *other* professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|--------|------------------|----------------------|-----------|
| PI: | Edward E. Max | Commissioned Officer | LIG/NIAID |
| OTHER: | Steven Esworthy | Staff Fellow | LIG/NIAID |
| | Ronald Lieberman | Commissioned Officer | LIG/NIAID |
| | Jeffrey Gimble | Medical Staff Fellow | LIG/NIAID |

COOPERATING UNITS (if any)

Stanley Korsmeyer, Metabolism Branch; David Sachs, Susan Epstein and Christian DeVaux, Immunology Branch, National Cancer Institute

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetic Research Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

3.7

PROFESSIONAL:

2.7

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
☐ (a1) Minors
☐ (a2) Interviews
☐ (b) Human tissues
☒ (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are investigating the regulation of expression of genes in the rabbit kappa (k) immunoglobulin (Ig) locus. Domestic rabbits harbor Ig k sequences that give rise to two intense bands when genomic Southern blots are probed with a fragment of the cloned klb4 gene. We have now cloned a k2b4 gene and a klb5 gene; accumulated evidence derived from all three genes identifies the two intensely hybridizing Southern blot bands as nominal expressed k isotype gene and a k2 isotype gene. The k2 gene is apparently not expressed in domestic rabbits, although there are no obvious defects in the coding sequences of the cloned k2b4 gene. The possibility that differential k1 vs k2 expression results from different regulatory sequences in the Jk-Ck intron is currently being explored by inserting these intron sequences into expression vectors to test their effects as enhancers of k gene transcription. In an attempt to understand latent k allotype expression in rabbits, we examined Southern blots of DNA from a rabbit in our colony that expressed latent allotype; no "extra" Southern blot bands that might have encoded latent allotype were observed.

Our studies of gene activation in human B cell development have shifted emphasis from the attempts to advance developmental stages of B cell lines in vitro to studies of the chromosomal state of regulatory regions, especially in the human k gene. We plan to apply the high-resolution Southern blot technology developed for genomic DNA sequencing to examine DNAase hypersensitivity sites in the human k enhancer and to look for differences between k-expressing and non-expressing cell lines with respect to DMS protection (footprinting) and Exonuclease III susceptibility in this region.

| | | |
|--|----------------------|---------------------------------------|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00180-06 LIG |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders) Interspecies and Human-Human Hybridomas and Monoclonal Antibodies | | |
| PRINCIPAL INVESTIGATOR (If not other nonprofessional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: John A. Sogn Research Chemist LIG/NIAID | | |
| OTHER: Xuan-Mao Li Visiting Fellow LIG/NIAID Henrietta Kulaga Staff Fellow LIG/NIAID | | |
| COOPERATING UNITS (if any) G. Marti, Hematology Branch, Clinical Center, NIH. | | |
| LAB/BRANCH Laboratory of Immunogenetics | | |
| SECTION Immunogenetics Research Section | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MAN-YEARS: 2.1 | PROFESSIONAL: 1.6 | OTHER: 0.5 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <p> Studies with the monoclonal antibody (McAb) McAb 33.1 have been proceeding in two directions. The first subject of investigation has been the exact nature of the human class II major histocompatibility antigen (MHC) recognized by McAb 33.1. It has been shown previously that the antigen is encoded by the HLA-DC subregion, but the degree of heterogeneity of antigens from this subregion is as yet unclear. McAb 33.1 has been compared to two other DC-specific antibodies, McAb Genox 3.53 and McAb Leu 10. The investigation has shown quite clearly that McAb 33.1 and McAb Leu 10 recognize the same determinant. McAb Genox 3.53 recognizes a distinct determinant. Clearance experiments suggested that the Genox 3.53 determinant is present on only a subset of the 33.1-positive molecules, raising the possibility of molecular heterogeneity. However, structural studies of the separated pools of antigen have failed to demonstrate any primary structural differences between the Genox 3.53-positive and Genox 3.53-negative fractions. The current hypothesis is that the adsorption differences reflect postsynthetic modifications or artifacts of isolation. The second subject under investigation is the enhancement of class II gene transcription upon B cell activation. B cells in various states of activation are being examined by the technique of Northern blotting to determine the degree of transcription of each individual class II MHC gene and to correlate specific enhancement of transcription with specific functional events. </p> | | |

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00352-02 LIG

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of Cell Surface Molecules Important for Immune Function

PRINCIPAL INVESTIGATOR (If other nonprofessional personnel below the Principal Investigator, (Name, title, laboratory, and institute affiliation))

PI: John E. Coligan Senior Investigator LIG/NIAID

OTHER: Ana Maria Diaz Primerano Visiting Fellow LIG/NIAID
Elliot Cowan Staff Fellow LIG/NIAID

COOPERATING UNITS (if any)

C. Terhorst, Dana-Farber Cancer Center; T.-H. Lee, Harvard School of Public Health;
R. Nairn, University of Michigan.

LAB/RANCH

Laboratory of Immunogenetics

SECTION

Membrane Antigen Structure Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

3.5

PROFESSIONAL:

2.0

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Multiple molecules exist on the surface of lymphocytes which are important for the development of the immune response. A major goal of these studies is to identify and structurally characterize these membrane-bound molecules, especially those present on cytotoxic T lymphocytes. Concordantly, it is important to have an understanding of the molecules encoded by infectious agents which are recognized by the immune system. Thus, the nature of the antigens in several viruses posing serious health problems is being investigated. Project areas include: (1) studies on the primary structure of the human T cell molecules T3 and T8; (2) preparation of mouse-human T cell hybrids in order to try to obtain a stable source of human T cell molecules; (3) characterization of the molecules of antigenic importance in human T cell leukemia viruses (HTLV); and (4) studies on antigenic variation in Herpes simplex virus type 1 (HSV-1) glycoproteins.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00389-01 LIG

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Studies of Human MHC Antigens in Recombinant Families

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|--------|-------------------|--------------------|-----------|
| PI: | Mary Ann Robinson | Staff Fellow | LIG/NIAID |
| OTHER: | Eric O. Long | Visiting Scientist | LIG/NIAID |
| | Thomas J. Kindt | Chief | LIG/NIAID |

COOPERATING UNITS (if any)

A. Johnson, and B. Hartzman, Georgetown University and Bernard Amos, Duke University.

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

2.0

PROFESSIONAL:

1.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- | | | |
|---|--|---|
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input checked="" type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors | | |
| <input type="checkbox"/> (a2) Interviews | | |

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The genetic organization of the HLA complex was analyzed by studying genomic DNA restriction enzyme polymorphisms in eight families that were known to include individuals inheriting recombinant HLA haplotypes. Cloned DNA corresponding to class I, class II (DR α , DR β , DC α , DC β and SB β) and class III (C4) major histocompatibility complex genes were used as probes in Southern blotting analysis. Polymorphism in the size of restriction fragments from the various major histocompatibility genes were observed with certain enzymes and could be assigned to haplotypes in families. The results to date have made it possible to: (1) identify a previously undetected crossover event in one family; (2) to localize the gene encoding C4 within the HLA complex between HLA-B and HLA-DR; (3) to distinguish HLA-DR-DC-SB haplotypes that type identically and, (4) to more precisely define the location of the crossover event in each recombinant haplotype.

LABORATORY OF IMMUNOLOGY
1984 Annual Report
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PHS-NIH
Summary Statement
Office of the Chief
Laboratory of Immunology
October 1, 1983 through September 30, 1984

Introduction

The Laboratory of Immunology is concerned with the elucidation of the fundamental mechanisms underlying immunologic responses. It has made rapid progress through the use of three new technologies which are creating a revolution in immunologic science. These are the use of monoclonal antibodies, the adaptation of techniques of molecular genetics to immunologic problems, and the use of long-term lines of cloned normal and transformed lymphocytes. The continued use and major improvement of these approaches should allow solution of many of the major problems which have concerned immunologists and should provide important advances in the efforts to more precisely regulate the normal and the disordered immune response.

Cloning of the Gene for the β Chain of the T Cell Receptor for Antigen

Laboratory of Immunology scientists have obtained cDNA clones for one of the constituent polypeptide chains of the T cell's antigen-binding receptor. This was achieved through the use of the subtractive cDNA cloning technology developed in previous years in the Laboratory and by taking advantage of a series of postulated properties of T cell receptor genes. These properties were: 1) expression in T cells but not B cells; 2) enrichment of mRNA for receptor in membrane associated polysomes; 3) rearrangement of receptor genes in the course of T cell differentiation; 4) translation from an mRNA of greater than 1 kb in size. The strategy used was to prepare a cDNA probe from the mRNA obtained from membrane-bound polysomes of a T cell hybridoma. This cDNA was then exhaustively hybridized with mRNA from a B lymphoma. The single stranded cDNA obtained represents mRNAs found in T cells and not B cells and associated with membrane bound polysomes. This cDNA was used to screen a cDNA library prepared from cDNA obtained by a subtractive T-B hybridization, using as sources of mRNA a T cell hybridoma and a B lymphoma which were different from those used to construct the cDNA probe. A group of positive cDNA clones were picked and tested for T cell "specificity" by Northern analysis. Those which hybridized with mRNAs of 1 kb or larger found in T cells, but not B cells, were selected for further study. One of these cDNA clones, when used in Southern analysis, hybridized with restriction fragments of different length in liver DNA and in DNA from T cell hybridomas. Moreover, each antigen-specific, class II-MHC molecule restricted T cell hybridoma examined proved to have a unique restriction fragment. A full length cDNA was obtained from a thymocyte library and sequenced. It proved to have statistically significant homology to immunoglobulin V and C region genes. Furthermore, when the sequence of this initial clone was compared to other cDNA clones from the thymocyte library, the 3'

(or C terminal) portions appeared identical while the 5' (or N terminal) portions were very different. Based on comparison with a partial sequence of a human receptor polypeptide, it was concluded that this gene encodes the β chain of the mouse T cell receptor for antigen. Studies aimed at proving this by examining the role of this molecule in T cell antigen-recognition are now in progress (Hedrick, Nielsen, Cohen, and Davis, LI/NIAID [Dr. Hedrick is now at the University of California, San Diego and Dr. Davis is at Stanford University]).

Analysis of rearrangement of T cell receptor gene in suppressor hybridomas

Suppressor T cells appear to have antigen-recognition characteristics which are quite distinct from helper and cytotoxic T cells. Both helper and cytotoxic T cells recognize antigen together with major histocompatibility gene products, referred to as co-recognition elements. By contrast, suppressor T cells have been reported to be capable of binding free antigen. The cloning of the gene for the β chain of the receptor of T cells which display histocompatibility restriction makes possible an examination of whether suppressor cells make use of the same chain in their antigen-binding receptor. A panel of more than 16 distinct T cell-T lymphoma hybridomas with suppressor function was assembled and subjected to Southern blotting analysis with a β chain probe. Three separate patterns of hybridization were seen in these experiments: 1) complete loss of genes encoding the β chain; 2) retention of germ line forms of these genes without appearance of rearrangements; 3) appearance of rearranged genes. Of the panel of suppressor T cell hybridomas, only two displayed rearrangement in β chain genes. These results suggest that at least some forms of suppressor T cells do not use the β chain in construction of their antigen-binding receptors. It seems likely that these suppressor T cells will use an analogous chain, encoded by a distinct but related gene, to form their antigen-binding receptors (Germain and Hedrick, LI/ NIAID [Dr. Hedrick is now at the University of California, San Diego]).

Chemical Properties of the Antigen-Binding Receptor of the T Cell Hybridoma 2B4

Monoclonal antibodies to unique antigenic determinants of receptors of individual T cell clones have been prepared and have made possible an analysis of the chain structure of the T cell receptor. Laboratory of Immunology scientists have prepared a monoclonal antibody which recognizes the receptor of the T cell hybridoma 2B4, which is specific for pigeon cytochrome *c* and which co-recognizes a class II MHC molecule. Based on immunoprecipitation of surface labelled molecules, it was shown that the receptor was a heterodimeric protein 85-90 kd in size, consisting of two chains of 45-50 kd and 40-44 kd, respectively. Isoelectric focusing studies indicate that an acidic chain (the α chain) is linked to one of two forms of the β chain. The two forms of β chain appear to reflect

different degrees of glycosylation. Indeed, treatment with endoglycosidase F reduces the α chain and the two forms of the β chain each to a core size of 33 kd. Studies of the migration pattern on sodium dodecyl sulfate-polyacrylamide gel electrophoresis in the presence or absence of reducing agents suggest that the receptor possess intrachain as well as interchain disulfide bonds. Cross-linking experiments indicate that the receptor is associated on the membrane with a 20-25 kd molecule, possibly analogous to the human T3 molecule. These analyses of the T cell receptor will help to provide the basis for a more complete understanding of the chemical basis of antigen-recognition by T cells (Samelson and Schwartz, LI/NIAID).

Use of Class I/Class II Recombinant Histocompatibility Genes to Analyze Domain Function

Class I and Class II major histocompatibility complex (MHC) molecules are membrane glycoproteins which express critical roles as co-recognition elements in T cell recognition and response to antigen on cell surfaces. Class I molecules are 45 kd polypeptides which are expressed on the cell membrane in a non-covalent complex with β_2 -microglobulin. Class II molecules consist of two chains (α and β) both encoded in the MHC. The expression of the β chain on the cell membrane appears to require that it interact with an α chain. Laboratory of Immunology scientists have begun an analysis of the structural basis of the interactions which are critical to such membrane expression. To do this, recombinant genes have been constructed which contain the 5' flanking region, the leader exon, and the first main coding block (β_1) of the gene for the β chain of the I-A^k class II molecule. These are covalently linked to the exon encoding the third external (C2) domain of the H-2D^d (or of the H-2L^d) class I molecule with the remaining 3' part of the H-2 gene. Since the C2 domain had been postulated to be the β_2 microglobulin binding portion of the class I molecule, it was reasoned that such a construct might be expressed on the cell membrane of transfected cells. It was found that pA^kC2D^d, the recombinant gene containing the β_1 domain of A^k and the C2 domain of H-2D^d was expressed on the cell surface of transfected L cells in non-covalent association with β_2 -microglobulin. This strongly supports the concept that the C2 domain is critical to β_2 -microglobulin binding and cell surface expression. Furthermore, it implies that the interactions are based on domain rather than whole molecule functions. The second recombinant gene, pA^kC2L^d, was actively transcribed in transfected cells but was not expressed on the cell surface. In view of previous findings that H-2L^d class I molecules bind β_2 -microglobulin less well than H-2D^d molecules, this result remains consistent with the concept that surface expression of class I and class II genes requires specific chain assemblages and that the C2 domain of class I polypeptides is critical to the interaction of that chain with β_2 -microglobulin (McCluskey and Margulies, LI/NIAID).

Structure-Function Analysis of Class II Molecules as Co-recognition Elements

Class II major histocompatibility complex (MHC) molecules are co-recognized with antigen by helper and other L3T4⁺ T cells. Furthermore, these molecules are the products of immune response genes and, as such, determine whether T cells can or cannot recognize a given antigen. This indicates that the structure of class II molecules is critical to the capacity to present specific antigens. To study the structural basis of the function of class II MHC molecules, Laboratory of Immunology scientists have utilized gene cloning, sequencing, and transfection techniques. Two major types of mouse class II molecules (I-A and I-E) exist and each is a heterodimer consisting of α and β chains, both encoded in the MHC. The cloning of a murine A_B cDNA was previously described and this cDNA was used to obtain genomic A_B genes of the b,d and k allelic forms, which have been sequenced. Others have cloned and sequenced A_α , E_β , and E_α genes and genomic clones for A_k , E_β and E_α are now available in the Laboratory of Immunology. To demonstrate unequivocally that particular class II molecules had specific antigen-presenting functions, plasmid vectors have been constructed allowing transfection and selection in both B lymphoma cells and Ltk fibroblastoid cells. Transformants were selected by electronic cell sorting based on the appearance of specific class II antigenic determinants on the transformed cells. Ltk cells cotransfected with A_B or $A_k A_k$ genes acquired antigenic determinants encoded by these genes and were able to present specific antigens, corecognized with I-A^d or I-A^k class II molecules, to T cell hybridomas. Exon shuffling experiments indicate that the 5' extracellular domain of the β chain (the β_1 domain) is very important in antigen-presenting function. This is consistent with the known high degree of allelic polymorphism of the β_1 domain compared to the β_2 domain. Cotransformation of L-cells with A_k and A_B or with A_B and A_k results in transfectants which failed to express membrane class II molecules despite the fact that they produce considerable amounts of mRNA. Since homologous α and β genes ($A_k A_k$; $A_B A_B$) are well expressed, these results indicate an unexpectedly strong control of chain assembly by the polymorphic regions of the α and β chain (Norcross, Bently, Braunstein, and Germain, LI/NIAID).

A correlated approach to the structure-function analysis of class II genes has been to develop serologic mutants by mutagenesis and antibody mediated selection of antigen-presenting cell lines. These mutants have been shown to have altered antigen presenting functions. In the cases examined thus far, the apparent mutation has involved a determinant on the β chain of the I-A^k class II molecule and the resultant cells have lost the capacity to present antigen to some, but not all, T cells which are cospecific for antigen and I-A^k class II molecules. Genomic clones of the I-A^k β_1 domain of the wild type cell line (TA3) and three mutant lines (A19, B18, and B13) have been obtained. The sequence of the TA3 β_1 domain was identical to that previously reported, while A19 and B18 had a single nucleotide

substitution leading to a glutamic acid to lysine change at position 67 in the β_1 domain, which is a highly polymorphic region. The sequence of B13 is in progress and each of the mutant β_1 domains have been engineered into transfection vectors so that the role of the identified mutation can be verified. These results indicate that single amino acid changes in class II molecules can profoundly alter their co-recognition function. They point the way to the detailed analysis of the structural basis of antigen presentation (Brown, Paul, and Germain, LI/NIAID; Glimcher, Harvard School of Public Health).

Molecular Events in Antigen-Processing

Most T cells recognize antigenic determinants in association with cell surface "co-recognition" elements, which are either class I or class II major histocompatibility complex molecules. Helper T cells and other L3T4⁺ T cells utilize class II molecules as such corecognition elements. Studies on the minimal antigenic structure required for T cell stimulation strongly suggest that the antigenic determinants recognized, together with class II molecules, by T cells are found on peptides cleaved from the native molecule and often displaying a three dimensional structure quite different from that which it has in the native state. Much of the evidence underlying this concept has come from studies of Laboratory of Immunology scientists on T cell responses to cytochrome c. It has recently been shown that antigen-presenting cells lightly fixed with paraformaldehyde can present enzymatic or chemical fragments of antigen, but not the intact molecule. This strongly implies that such cells have lost the ability to "process" antigen but remain competent to present already "processed" peptides. During the past year, Laboratory of Immunology scientists have analyzed certain aspects of the structural basis of antigen processing in the cytochrome c system. The principal determinant recognized by cloned T cells is found on the cyanogen bromide fragment consisting of amino acids 81-104 and this peptide is efficiently presented by fixed cells. In contrast, apocytochrome c and the acid hydrolysis fragment spanning amino acids 60-104 are not presented by fixed antigen-presenting cells, although intact antigen-presenting cells present these molecules efficiently. The fragment consisting of amino acids 66-104, prepared by partial cyanogen bromide cleavage, could be presented by fixed cells indicating that removal of residues 60 to 65 was a critical event. An analysis of the three dimensional structure of cytochrome c suggests that the amino acid at position 61 (normally glutamic or aspartic acid) interacts with lysine at position 99 to stabilize the interaction of helices formed by residues 62-70 and the C terminal residues 88-101. Removal of residues 60 to 65 would eliminate this interaction and, in particular, free the lysine at position 99, already shown to be the most likely residue to contact the T cell receptor, for its interaction with the T cell. This provides perhaps the most detailed understanding currently available for the formation and specificity of T cell specific epitopes (Kovac and Schwartz, LI/NIAID).

Accessory Cell Requirements for Induction of Receptors for Interleukin-2

The proliferation of activated T lymphocytes is dependent on the action of a hormone-like polypeptide designated interleukin-2 (IL-2). IL-2 acts by binding to a membrane receptor expressed on suitably activated, but not resting, T lymphocytes. Laboratory of Immunology scientists have prepared a group of monoclonal antibodies specific for distinct epitopes on the IL-2 receptor. One of these, 3C7, competitively inhibits the binding of ^3H -IL-2 to the T cell and inhibits T cell responses to IL-2. A second antibody, 7D4, is a potent inhibitor of IL-2 stimulation of T cells but does not block binding of IL-2 to the receptor, while the third antibody, 2E4, only weakly inhibits T cell activation in response to IL-2 and does not block binding of IL-2. The availability of these highly specific reagents has allowed Laboratory of Immunology scientists to examine the requirements for induction of receptors for IL-2. Class I-restricted, $\text{Lyt}2^+$ T cells of the cytotoxic/suppressor phenotype expressed very substantial amounts of membrane receptors for IL-2 in response to concanavalin A (Con A) in the presence or absence of a source of accessory cells. In contrast, $\text{L}3\text{T}4^+$, $\text{Lyt}2^+$ T cells required the presence of accessory cells to develop their full complement of IL-2 receptors in response to stimulation with Con A. Macrophages, B lymphoma cells, fibroblasts, and certain functional T cell hybridomas could all serve as accessory cells for the induction of IL-2 receptors by $\text{L}3\text{T}4^+$ T cells. It was not necessary that the accessory cell population bear class II molecules. A series of soluble mediators, including interleukin-1, interleukin-2, interleukin-3, and γ -interferon failed to substitute for accessory cells but phorbol myristate acetate was effective as an accessory cell replacement. These studies suggest that growth control of $\text{L}3\text{T}4^+$ and $\text{Lyt}2^+$ T cells may be quite different and must be taken into account in efforts to propagate these cells either in vitro or in vivo. This information will also be of value in efforts to specifically manipulate growth of T cells of these distinct types (Malek, Ortega and Shevach, LI/NIAID).

A Monoclonal Antibody to Thy 1 Is a Potent T Cell Stimulant

Thy 1 is a membrane glycoprotein with amino acid sequence homology to immunoglobulin. It is found on mouse thymocytes, T lymphocytes, neurons, fibroblasts, and epithelial cells. Recent studies had suggested a possible role for Thy 1 in the transduction of activation signals in the process of T cell responses. Laboratory of Immunology scientists have undertaken an examination of this issue through the use of monoclonal antibodies to the Thy 1 molecule. One such monoclonal antibody, G7, has been obtained which proved to be a potent inducer of interleukin-2 (IL-2) production from all functioning T cell hybridomas as well as from normal T cells. Indeed, G7 proved to be a potent mitogen for normal T cells and was as effective as concanavalin A in inducing the expression of IL-2 receptors on T cells. This suggests that the interaction of G7 with its

epitope on the Thy 1 molecule initiates a series of events common to T cell activation mediated by many stimulatory ligands. Indeed, the Thy 1 molecule may be part of a final common pathway by which T cell activation mediated by any stimulus may proceed (Gunter and Shevach, LI/NIAID).

Structural and Genetic Basis for Expression of Latent Immunoglobulin Allotypes

Immunoglobulins from many species exist in alternative allelic forms. Among the best understood examples of such genetically determined polymorphism or allotypy are the κ chain and H chain variable region allotypes of rabbits. Several investigators have reported that, under special circumstances, rabbits may produce immunoglobulin of allotypes different from those expected from the genotype of the responding animals. This enigmatic and potentially very important phenomena has been examined by Laboratory of Immunology scientists.

Rabbit kappa chains are encoded by genes at the b locus. Animals which appear to be genetically homozygous for the b_9 allele (b_9/b_9) will, under certain forms of stimulation, produce small amounts of Ig which appear by serologic typing to be of the b_5 type. cDNA probes which distinguish b_5 and b_9 mRNAs have been developed; the b_5 cDNA was shown to be capable of detecting b_5 mRNA at the level of 0.1% of total κ chain mRNA. When RNA from splenic lymphocytes of animals producing serologically detectable b_5 were screened with the b_5 cDNA probe, no hybridization was observed. Furthermore, genomic DNA from b_9/b_9 rabbits fails to hybridize with b_5 cDNA by Southern blotting, indicating an absence of the b_5 gene. Thus, latent allotypy for the κ locus of rabbits in the Laboratory of Immunology colony must be based on some mechanism other than expression of authentic genes for such allotypes.

Studies of the structural basis of the V_H allotypy indicate that seven positions in the first framework region and ten positions in the third framework region of the V_H chain are allotype-correlated and that differences at this position may lead to serologically detectable allotypic determinants. Most of the alternative amino acids at these positions can be derived from each other by single base changes. Thus, somatic mutation or gene conversion-like events are possible mechanism for the production of latent V_H allotypes (Mage, Bernstein, McCartney-Francis, LI/NIAID).

Partial Purification of B Cell Stimulatory Factor (BSF)-p1.

The proliferation of resting B cells stimulated with low concentrations of anti-IgM antibodies is dependent upon a T cell derived factor which had been referred to a B cell growth factor (BCGF). This factor has recently been designated BSF-p1. Initial chemical characterization of BSF-p1 by Laboratory of Immunology scientists and their colleagues indicated that it had a molecular

weight of 14-15 kd and existed in two principal charged forms, with pI's of 6.4 and 7.4, respectively. Hydrophobic chromatography indicated that it could be cleanly separated from interleukin-2. During the past year, efforts to purify this factor have been undertaken. Ten to fifteen liter batches of EL-4 thymoma cells, in serum-free medium, have been induced with phorbol myristate acetate. Cell free medium was collected at 48 hours and proved to have considerable BSF-p1 activity (~800 units/ml). This material was initially allowed to adsorb to controlled-pore glass beads conjugated with trimethylsilyl groups and eluted with 50% acetonitrile. BSF-p1 bound quantitatively to the beads and could be eluted with good yield. BSF-p1 was further purified by sequential application to a C18 reverse phase column developed by high pressure liquid chromatography with 10-60% and 40-60% acetonitrile gradients, respectively. This partially purified material was used for immunization of rats in an effort to prepare monoclonal anti-BSF-p1 antibodies and for further purification. Several candidate BSF-p1 specific monoclonal antibodies are now under active evaluation (Ohara, Inman and Paul, LI/NIAID).

Interleukin 2 Has B Cell Differentiation Activity.

B cell stimulated with anti-IgM and the B cell growth factor BSF-p1 enter S phase and divide but fail to differentiate into high rate IgM synthesizing cells. Laboratory of Immunology scientists have previously shown that these stimulated cells will undergo differentiation in the presence of two additional T cell derived factors. One, B15-TRF, is found in the supernatant of the B15L12 T cell hybridoma and is required early in culture. The second, EL-TRF, is obtained from supernatants of EL-4 cells induced with phorbol myristate acetate. This stimulation to high rate IgM synthesis is accompanied by the induction of mRNA for the secretory form of the μ H chain and a greater than ten-fold change in the ratio of mRNAs for the secretory and membrane forms of μ . Associated with this was a striking induction in mRNA for the J chain and the appearance of J chain protein. J chain is required for the formation of polymeric IgM and its expression has been postulated to be co-regulated with that of the secretory form of the μ chain. None of these induction events are noted unless both B15-TRF and EL-TRF are added to cultures of B cells stimulated with anti-IgM and BSF-p1; this strengthens the contention for a critical role of a series of growth and differentiation factors acting in an ordered sequence to develop a strong IgM secretory response.

The chemical nature of EL-TRF has been subject to study during the past year. Sequential separations by gel filtration and sodium dodecyl sulfate-polyacrylamide gel electrophoresis indicates the existence of two entities in EL4 supernatant with EL-TRF activity, one 32 kd in size, the other 16 kd. The 16 kd material coelectrophoreses with interleukin 2 and its activity as a B cell differentiation factor is inhibited by a site-specific monoclonal antibody to the T cell

receptor for IL-2. By contrast, the activity of the 32 kd EL-TRF is not blocked by the monoclonal antibody. Molecularly cloned IL-2, at concentrations of 100 to 1000 units/ml expresses EL-TRF activity which is also blocked by anti-IL-2 receptor antibody. Support for the direct action of IL-2 on activated B cells comes from the demonstration that small amounts of ³H-IL-2 (~600 molecules/cell) bind specifically to activated B cells and that monoclonal antibodies to the IL-2 receptor bind to such B cells at a level of approximately 1% of their binding to the IL-2 dependent T cell line, HT-2. These results indicate that IL-2 may have wider physiologic effects than regulation of T cell growth and may play an important role in stimulation of Ig synthesis by B cells (Nakanishi, Cohen, Malek, Shevach and Paul, LI/NIAID; Blackman and Koshland, University of California, Berkeley; Smith, Dartmouth University; Hamaoka, Osaka University).

Honors, Awards and Scientific Recognition

Members of the Laboratory of Immunology play important roles in the national and international scientific community. They serve on editorial boards of many important journals. Dr. Paul is editor of the Annual Review of Immunology and is a member of the editorial boards of the Journal of Immunology, Immunological Reviews, the Journal of Molecular and Cellular Immunology, Human Immunology, and the Journal of Clinical Immunology. He is an advisory editor of the Journal of Experimental Medicine and an associate editor of Immunological Communications. Dr. Shevach is a section editor of the Journal of Immunology, a member of the editorial advisory board of the Journal of Molecular and Cellular Immunology, and a member of the editorial boards of Cellular Immunology, the Journal of Immunological Methods, and Proceedings of the Society for Experimental Biology and Medicine. Dr. Germain is an associate editor of the Journal of Immunology, a member of the editorial advisory board of the Journal of Molecular and Cellular Immunology, and a member of the editorial board of the Journal of the Reticuloendothelial Society. Dr. Schwartz is a member of the editorial board of the International Journal of Cell Cloning and of the editorial advisory board of the Journal of Molecular and Cellular Immunology. Dr. Green is a member of the editorial boards of Immunological Communications and of Clinical Immunology and Immunopathology. Dr. Mage is a member of the editorial board of the Journal of Immunologic Methods. Dr. Paul was the editor of two books, Fundamental Immunology and Immunogenetics, published this year by Raven Press and is the advisory editor for Immunological Diseases of the Cecil Textbook of Medicine.

Dr. Paul is a councillor of the American Association of Immunologists and a member of the Board of Directors of the Foundation for Advanced Education in the Sciences. He completed a term as chairman of the U.S. Immunology Board of the U.S.-Japan Cooperative Medical Sciences Program, as a member of the Scholars Selection Committee of the Fogarty International Center, and as a member of the Committee on Promotions and Tenure, NIAID. He is a member of the Scientific Review Board of the Howard Hughes Medical Institute, the Scientific Board of Visitors of the Oklahoma Medical Research Foundation, the Board of Scientific Advisors of the Jane Coffin Childs Memorial Fund for Medical Research, the Scientific Advisory Committee of the New England Regional Primate Center, the Scientific Review Committee of the Cambridge Branch of the Ludwig Institute, the Board of Scientific Consultants of the Memorial-Sloan Kettering Cancer Center, the Advisory Committee of the Harold C. Simmons Arthritis Research Center, and the International Steering Committee of the Maimonides Conferences on Cancer Research.

During the past year, Dr. Paul presented the Carl Prausnitz Lecture at the meeting of Collegium Internationale Allergologicum in Puerto Vallarta, Mexico, was an invited lecturer at the annual meeting of the German Immunological Society, a participant in the Dahlem Workshop on Leukemia in Berlin, a session chairman and speaker at the Ortho- UCLA Symposium on the Regulation of the Immune System, keynote lecturer at the Symposium in the Biomedical Sciences of the University of Washington, a session chairman and speaker at the Gordon Conference on Immunochemistry and Immunobiology and at the FASEB Conference on the Development and Senescence of the Immune System, a lecturer at the FASEB Conference on Receptors, and a plenary lecturer at the 10th International Congress of the Transplantation Society.

Dr. Schwartz was the recipient of a USPHS Commendation Medal. He chaired a mini-symposium at the annual meeting of the American Association of Immunologists, and was a session chairman at the FASEB Conference on Receptors and at the Gordon Conference on Immunochemistry and Immunobiology. He was an invited speaker at the Ortho- UCLA Symposium on the Regulation of the Immune System, and at the Arden House Conference on the Role of Histocompatibility Molecules in Biology.

Dr. Shevach is a member of the Research Committee and of the Fellowship Subcommittee of the Arthritis Foundation, and a member of the Program Committee of the American Association of Immunologists. He was a mini-symposium speaker at the annual meeting of the American Association of Immunologists and at the International Congress of Cell Biology in Tokyo. He was a session chairman and speaker at the International Congress on the Role of Accessory Cells in the Immune Response and at the Arthritis Foundation Fellowship Conference.

Dr. Mage is chairman of the Immunology Division of the American Society for Microbiology and is a member of the Executive Council of DC Chapter of Sigma Xi. She is a member of the NIH Animal Genetics Resource Advisory Committee and Coordinator for the Advanced Immunology Course sponsored by the Foundation for Advanced Education in the Sciences. She was an invited lecturer at the WHO Immunology Training Center course in Lausanne, Switzerland.

Dr. Germain is a member of the American Cancer Society Advisory Committee on Immunology and Immunotherapy. He chaired a workshop at the Ortho- UCLA Symposium on Regulation of the Immune System and a mini-symposium at the annual meeting of the American Association of Immunologists. He was an invited speaker at the Third H-2, HLA Cloning Meeting in Strasbourg, France.

Dr. Green is a member of the Immunohematology Committee of the American Society of Hematology, the NIAID Animal Care Committee and the NIAID Clinical Research Subpanel. He was an invited speaker at the Centre International de Recherches Dermatologiques Symposium on Immunodermatology in Valbonne, France.

Dr. Margulies was a session chairman at the Howard Hughes Medical Institute Symposium on Qa and T1a-Region Genes and was a speaker at the Third Annual H-2, HLA Cloning Meeting in Strasbourg, France.

Dr. Jonathan Ashwell presented a mini-symposium lecture at the annual meeting of the American Association of Immunologists and was an invited speaker at the FASEB Conference on Receptors. Dr. Thomas Malek and Dr. Zdenko Kovac were invited speakers at the Gordon Conference on Immunochemistry and Immunobiology. Dr. James McCluskey chaired a session and was an invited speaker at the Satellite Symposium on Congenital Adrenal Hyperplasia at Cornell University Medical College.

In addition, members of the Laboratory of Immunology presented research seminars and lectures at universities and research institutes both in the United States and abroad.

Administrative, Organizational and Other Changes

The Laboratory of Immunology continues to play an important role in the training of young scientists. During the past year, a group of outstanding individuals completed their postdoctoral training in the Laboratory. They included Kenneth Bernstein, Stephen Hedrick, Maureen Howard, James Jakway, Hyun Lillehoj, Kenji Nakanishi, Susumu Nishinarita, and Peter Wassmer. Each of these scientists made very substantial contributions to the research program of the Laboratory of Immunology. A series of post-doctoral fellows joined the Laboratory of Immunology for research training during the past year. Included among them were Samuel Breit, Linda Hillstrom, Mitsuo Honda, Richard Kroccek, Robert Lechler, Junichiro Mizuguchi, Franca Ronchese, and Wayne Tsang.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00030-16 LI

PERIOD COVERED

October 1, 1983 to September 30, 1984

*TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Antigen Recognition and Activation of Immunocompetent Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

| | | | |
|---------|--------------|---------------------|-----------|
| PI: | E. Paul | Chief | LI, NIAID |
| Others: | E. Shevach | Senior Investigator | LI, NIAID |
| | R. Germain | Senior Investigator | LI, NIAID |
| | K. Nakanishi | Visiting Fellow | LI, NIAID |
| | J. Ohara | Visiting Associate | LI, NIAID |
| | J. Mizuguchi | Guest Worker | LI, NIAID |
| | E. Rabin | Microbiol. Tech. | LI, NIAID |
| | M. Brown | Guest Worker | LI, NIAID |
| | T. Malek | Research Expert | LI, NIAID |
| | J. Miller | Guest Worker | LI, NIAID |

COOPERATING UNITS (if any)

Dartmouth University, Dartmouth, NH (K. Smith) and University of Osaka, Osaka, Japan (T. Hamaoka).

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

4.5

PROFESSIONAL

2.7

OTHER

1.8

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

The goal of this project is to study the control of B lymphocyte growth and differentiation by hormone-like polypeptides acting together with ligands which cross-link antigen-binding receptors. Resting B cells may be activated to enter the G₁ phase of the cell cycle by anti-IgM antibodies and can be stimulated to enter the S phase by the B cell growth factor, BSF-p1. BSF-p1 has been partially purified from a serum-free supernatant of a T lymphoma line by reverse phase high pressure liquid chromatography. Efforts to prepare monoclonal antibodies to BSF-p1 and to obtain cDNA clones for this growth factor are in progress.

B cells activated with anti-IgM and BSF-p1 will differentiate into high-rate IgM synthesizing cells under the influence of two T cell-derived differentiation factors, one [B15-TRF] required early in culture, and the other [EL-TRF] required late. This stimulatory scheme has been also shown to lead to the induction of mRNA for the secretory form of the μ H chain and for J chain. Interleukin-2, the T cell growth factor, has been demonstrated to have EL-TRF activity. In addition, a second T cell derived factor, not yet characterized, also has strong EL-TRF activity.

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| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00035-09 LI |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Specificity in Immune Responses | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: J. K. Inman Senior Investigator LI, NIAID | | |
| | | |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Immunology | | |
| SECTION | | |
| INSTITUTE AND LOCATION National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20205 | | |
| TOTAL MAN-YEARS: 1.4 | PROFESSIONAL: 1.0 | OTHER: 0.4 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The principal aim of this project is to test the hypothesis of general multi-specificity for the combining regions of antibodies and other kinds of receptors. Receptor sites, according to theory developed earlier in this project, should be capable of interacting with virtually any substance in a manner that will lower the standard free energy of the system and thus exhibit an equilibrium association constant greater than 1. Most associations will be weaker than ones commonly measured, but occasional substances may bind to a receptor with affinities high enough to affect biological function. Their structures may not necessarily resemble those of the recognized effector, substrate or antigen. </p> <p> The above hypothesis is being tested in the following way: Radiolabeled, monoclonal antibodies or solubilized receptors are passed through small, affinity chromatography columns. Accurate measurements are made of the retention (retardation) caused by a matrix-bound reference ligand in the presence and absence of many, diverse, suitably large compounds. The resulting retention values are employed directly in calculating association constants for these compounds and the receptor site. The distribution of constants provides a description of the receptor's multispecific character. The technique of quantitative affinity chromatography, developed in this study, provides a general and effective means for estimating very low to moderately strong association constants for antibodies and requires very small samples. </p> <p> Large affinity probes that can be covalently bound to affinity matrices have been synthesized by systematic strategies developed in an earlier phase of this project. Multispecific associations with such probes will be usefully employed in extending the scope of specific, affinity-based separations. Knowledge of multispecificity frequencies should play an essential role in understanding specificity (selectivity) in biological recognition and control. Special attention will be given to applying these findings to models of immune systems and control networks. </p> | | |

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|--|---|--|---|--|---|--------------------------------------|---------|-----------|--|-----------|--|----------------------|--------------|-----------|--|--------------|----------------------|-----------|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00036-19 LI | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Ig Genetics: Ontogeny & Differentiation of Cells of the Rabbit Immune System | | | | | | | | | | | | | | | | | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 40%;">R. G. Mage</td> <td style="width: 30%;">Senior Investigator</td> <td style="width: 15%;">LI, NIAID</td> </tr> <tr> <td>Others:</td> <td>E. Lamoyi</td> <td>Visiting Associate</td> <td>LI, NIAID</td> </tr> <tr> <td></td> <td>N. McCartney-Francis</td> <td>Guest Worker</td> <td>LI, NIAID</td> </tr> <tr> <td></td> <td>K. Bernstein</td> <td>Medical Staff Fellow</td> <td>LI, NIAID</td> </tr> </table> | | | PI: | R. G. Mage | Senior Investigator | LI, NIAID | Others: | E. Lamoyi | Visiting Associate | LI, NIAID | | N. McCartney-Francis | Guest Worker | LI, NIAID | | K. Bernstein | Medical Staff Fellow | LI, NIAID |
| PI: | R. G. Mage | Senior Investigator | LI, NIAID | | | | | | | | | | | | | | | |
| Others: | E. Lamoyi | Visiting Associate | LI, NIAID | | | | | | | | | | | | | | | |
| | N. McCartney-Francis | Guest Worker | LI, NIAID | | | | | | | | | | | | | | | |
| | K. Bernstein | Medical Staff Fellow | LI, NIAID | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) University of Alabama, Birmingham, AL (W. E. Gathings); Basel Institute of Immunology, Basel, Switzerland (A. S. Kelus); and LMB, NIADDK (G. A. Cohen, E. Padlan & D. Davies). | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Immunology | | | | | | | | | | | | | | | | | | |
| SECTION | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20205 | | | | | | | | | | | | | | | | | | |
| TOTAL MAN-YEARS <div style="text-align: center; font-weight: bold;">2.9</div> | PROFESSIONAL <div style="text-align: center; font-weight: bold;">1.5</div> | OTHER <div style="text-align: center; font-weight: bold;">1.4</div> | | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td><input type="checkbox"/> (a) Human subjects</td> <td><input type="checkbox"/> (b) Human tissues</td> <td><input checked="" type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td><input type="checkbox"/> (a2) Interviews</td> <td></td> <td></td> </tr> </table> | | | <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input checked="" type="checkbox"/> (c) Neither | <input type="checkbox"/> (a1) Minors | | | <input type="checkbox"/> (a2) Interviews | | | | | | | | | |
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input checked="" type="checkbox"/> (c) Neither | | | | | | | | | | | | | | | | |
| <input type="checkbox"/> (a1) Minors | | | | | | | | | | | | | | | | | | |
| <input type="checkbox"/> (a2) Interviews | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> We investigated the cellular expression of Ig allotypes, their altered phenotypic expression in cultured cells and in mutant Basilea rabbits that produce elevated levels of λ type light chains and an unusual K2 isotype. We showed that the sire of the Basilea mutant expressed two isotypic forms of κ light chains (K1,b9 and K2,bas) and that the mutant lost the trait of expression of normal K1,b9 protein. The DNA of the mutant rabbits still contains structural genes corresponding to contant regions of both K1,b9 and K2,bas isotypes. Although K1,b9 and K2,bas are probably closely linked structural genes, the trait of expression of the K2,bas isotype maps in our breeding studies as an allele at the b locus; no recombinants have been observed yet. Immunofluorescence studies of Basilea rabbits suggest that a b9-like light chain is produced by pre-B cells but not by plasma cells. We hypothesize that there is a structural defect in this light chain that diminishes or destroys its ability to function as part of an intact Ig. </p> <p> Allotype-specific probes were used to search for latent b5 allotype in the expressed mRNAs of b9 splenocytes that were cultured with LPS and anti-b9 in order to modulate their expression of κ light chains. The sensitivity of our original dot blot tests was increased in Northern blotting and S1 protection experiments yet no RNA with b5-encoding sequences was detected. Moreover, when the allotype-specific b5 probe was used in Southern analyses of DNAs from b9,bas and b5 animals, it detected the K1,b5 gene in b5 DNA only. Alternative explanations for the apparent expression of latent kappa allotypes at the protein level must be considered. </p> <p> We reexamined earlier protein sequence data in the light of our newly deduced protein sequences from cloned DNAs to obtain new insight into the structural basis for VHα allotypes. The allotypes differ at seven positions in framework one and ten positions in framework three; most alternative amino acids can be derived from each other by single base changes. We suggest that some reported latent VHα allotypes may have arisen by somatic mutations and gene-conversion-like events that altered germ-line genes for nominal allotypes. </p> | | | | | | | | | | | | | | | | | | |

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00037-17 LI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunogenetics of Mouse Immunoglobulins and Genetic Control of Antibody Response

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R. Lieberman Senior Investigator LI, NIAID

Others: S. Nishinarita Visiting Fellow LI, NIAID

COOPERATING UNITS (if any) University of Michigan Medical School, Ann Arbor, MI (J. L. Claflin); Karolinski Institutet, Stockholm, Sweden (G. Klein and F. Wiener); and Washington University School of Medicine, St. Louis, MO (J. M. Davie & A. M. Staniz).

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:

3.0

PROFESSIONAL:

2.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Antibody diversity may arise by random combination of VH-D-J and VL genes and by somatic mutation. In this regard amino acid sequences and serologic identification of idiotypes (Id) of structurally and functionally related antibodies have been extensively studied. Phosphoryl choline (PC) binding myeloma proteins (MP) and hybridoma proteins (HP) have been especially useful in studies of Id heterogeneity. In the mouse at the DNA level studies of PC binding MP or HP show that the entire VH repertoire is generated from a T15 germ line gene and that somatic mutations account for the VH variations in M167 or M603 MP. Based on sequence studies, VL regions of PC binding MP fall into three kappa sub-groups: T15 (VK22), M167 (VK24) and M603 (VK8). PC antibodies are constructed by association of any of these light chains with the VH₄ (T15 heavy chain germ line sequences). Serologically defined idiotopes have been identified for each group. One of the more complex problems is the relationship of the primary amino acid sequence to the conformational structure in the expression of an idiotope. In the studies presented we prepared monoclonal anti C3Id antibodies (NL-16 and NL-24). C3Id is associated with a CBBPC3 MP from a C.BB₂₂ mouse and is identical in amino acid sequence to T15 MP except for four VH framework amino acids and a different constant region. NL16 antibody identified an Id (C3-16) determined by the D-region (YYGSS) and the VK22 L-chains of T15. C3-16Id was found to be associated with the PC combining site suggesting that the D-region conformational structure and light chains effected the Id function. NL-24 antibody identified an Id (C3-24) present on C3, T15, and H8 but not on light chain unrelated M603 or M167 PC binding MP. Only T15Id⁺ IgA and not IgG or IgM PC binding MP or HP expressed C3-24 Id. However, T15Id⁺ HP did not express C3-24 Id. This C3-24 Id requires T15IdX (VK22-VH4) and IgA CH regions to be expressed in PC binding antibodies. Anti PC serum from a variety of inbred strains did not inhibit C3-24. However, when IgA fraction was isolated from a pool of anti PC serum of BALB/c mice, 70% inhibition of NL-24 and C3 binding occurred.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00040-08 LI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Genetic control of immunocompetent cell interactions

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Ethan M. Shevach, M.D., Sr. Investigator, LI, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD

TOTAL MAN-YEARS

PROFESSIONAL

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been terminated.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00147-09 LI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Mechanism of Activation of Thymus-Derived Lymphocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R. H. Schwartz Senior Investigator LI, NIAID

| | | | |
|---------|--------------|--------------------|-----------|
| Others: | L. Samelson | Research Expert | LI, NIAID |
| | H. Narimatsu | Visiting Associate | LI, NIAID |
| | Z. Kovac | Visiting Fellow | LI, NIAID |
| | B. Fox | Guest Worker | LI, NIAID |

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:

4.0

PROFESSIONAL:

3.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to understand the biochemical basis of antigen-specific T cell activation and the role played by immune response genes in the regulation of this event. During the past year we have used a monoclonal antibody against the T cell antigen-specific receptor on an MHC-restricted T cell hybridoma to isolate and characterize the receptor. The molecule is a glycoprotein composed of two different polypeptide chains, each of approximately 40,000-44,000 daltons in molecular weight, that are linked together by disulfide bonds. Both chains have a core size of about 33,000 daltons after removal of sugars with endoglycosidases and appear to contain intrachain disulfide bonds. The chains differ in their isoelectric points and thus can be separated on the basis of charge. One chain was found to exist in two different molecular weight forms apparently due to differences in the level of glycosylation.

In a separate set of experiments we undertook a comparison of two different mechanisms of Ir gene control, thymic selection and antigen-presentation, to determine which was dominant in the regulation of the pigeon cytochrome c T cell proliferative response of [B10.A(4R)xB10.PL]F₁ mice. Using radiation-induced bone marrow chimeras it was possible to dissect out these two effects and show that the quantitative decrease in Ia molecule expression on the surface of the antigen-presenting cell was sufficient to explain the nonresponsiveness of the F₁.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00148-09 LI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Identification of the TSTA of the L2C Guinea Pig Leukemia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: I. Green

Senior Investigator

LI, NIAID

COOPERATING UNITS (if any)

Dermatology Branch, NCI (V. Hearing)

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

0.8

PROFESSIONAL

0.4

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The L₂C leukemia is a B cell leukemia of inbred strain 2 guinea pigs. These cells have surface IgM and C3 receptors. Studies have shown that these leukemia cells possess a strong tumor specific transplantation antigen (TSTA) that can easily be demonstrated by immunization protection tests in syngeneic animals. A procedure employing KCl extraction of the leukemic cell yields a soluble extract that is also highly antigenic. The physical and chemical properties of this soluble TSTA are now the subject of study. The findings to date indicate that this TSTA has several unusual properties; it has a M.W. of 12,500, (as determined by Sephadex chromatography and polyacrylamide gel electrophoresis); it is resistant to boiling for 5 minutes as well as to extremes of pH. Iso-electric focusing indicates that the immunogenic material has a high isoelectric point, between 9-10. Treatment with trypsin, neuraminidase and periodate destroys the activity. The fraction containing the TSTA was pink suggesting that it might be cytochrome c. However, immunization with mouse cytochrome c (having the same sequence as guinea pig cytochrome c) failed to protect. Furthermore, fractionation of cells demonstrated that fractions containing membranes protected while the cytosol soluble fraction failed to protect. These results suggest that the TSTA is not cytochrome c but that it is another small, basic glycoprotein.

| | | | | | | | | | | | | | | | | | | | | | | |
|--|----------------------|--|-----------|----------------|---------------------|-----------|---------|----------|-----------------|-----------|--|-----------|-----------------|-----------|--|------------|----------------------|-----------|--|----------|--------------|-----------|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00223-03 LI | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Cellular Interactions in the Immune Response | | | | | | | | | | | | | | | | | | | | | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">R. H. Schwartz</td> <td style="width: 35%;">Senior Investigator</td> <td style="width: 15%;">LI, NIAID</td> </tr> <tr> <td>Others:</td> <td>Z. Kovac</td> <td>Visiting Fellow</td> <td>LI, NIAID</td> </tr> <tr> <td></td> <td>G. Suzuki</td> <td>Visiting Fellow</td> <td>LI, NIAID</td> </tr> <tr> <td></td> <td>J. Ashwell</td> <td>Medical Staff Fellow</td> <td>LI, NIAID</td> </tr> <tr> <td></td> <td>H. Quill</td> <td>Staff Fellow</td> <td>LI, NIAID</td> </tr> </table> | | | PI: | R. H. Schwartz | Senior Investigator | LI, NIAID | Others: | Z. Kovac | Visiting Fellow | LI, NIAID | | G. Suzuki | Visiting Fellow | LI, NIAID | | J. Ashwell | Medical Staff Fellow | LI, NIAID | | H. Quill | Staff Fellow | LI, NIAID |
| PI: | R. H. Schwartz | Senior Investigator | LI, NIAID | | | | | | | | | | | | | | | | | | | |
| Others: | Z. Kovac | Visiting Fellow | LI, NIAID | | | | | | | | | | | | | | | | | | | |
| | G. Suzuki | Visiting Fellow | LI, NIAID | | | | | | | | | | | | | | | | | | | |
| | J. Ashwell | Medical Staff Fellow | LI, NIAID | | | | | | | | | | | | | | | | | | | |
| | H. Quill | Staff Fellow | LI, NIAID | | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) None | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Immunology | | | | | | | | | | | | | | | | | | | | | | |
| SECTION | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20205 | | | | | | | | | | | | | | | | | | | | | | |
| TOTAL MAN-YEARS: 5.0 | PROFESSIONAL: 4.0 | OTHER: 1.0 | | | | | | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> This project attempts to understand the biochemical basis for T cell interactions with other cells in the immune system. During the past year we have made progress in two areas. First, studies of antigen-processing, using paraformaldehyde fixed B cell tumors and the antigen pigeon cytochrome c, have shown that this molecule must be cleaved to a fragment consisting of residues 66-104 in order for it to be stimulatory for T cells. Fragment 60-104 was non-stimulatory. Analysis of the xray structure of the molecule suggests that this might be because a negatively charged glutamic acid at position 61 normally interacts with the lysine at position 99, a residue we have previously shown is critical for T cell activation. When residues 60 to 65 are eliminated, the lysine at 99 is freed and can then interact with the T cell receptor. </p> <p> In the second area, we have discovered an unusual T cell population in low responder animals which can be activated by a 17 amino acid peptide of pigeon cytochrome c in association with the low responder Ia molecule, but which is blocked from responding when the intact cytochrome c molecule is present. Surprisingly, the blocking appears to be noncompetitive. The relationship of this unusual clone to the low responder phenotype of the animal is currently being investigated. </p> | | | | | | | | | | | | | | | | | | | | | | |

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00224-03 LI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Monoclonal Antibodies as Probes for T Cell Activation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

| | | | |
|---------|---------------|----------------------|-----------|
| PI: | E. M. Shevach | Senior Investigator | LI, NIAID |
| Others: | T. Malek | Research Expert | LI, NIAID |
| | H. Lillehoj | Staff Fellow | LI, NIAID |
| | L. Logdberg | Visiting Fellow | LI, NIAID |
| | K. Gunter | Medical Staff Fellow | LI, NIAID |
| | G. Ortega | Guest Worker | LI, NIAID |
| | M. Honda | Guest Worker | LI, NIAID |
| | R. Kroccek | Guest Worker | LI, NIAID |

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

7.5

PROFESSIONAL:

5.0

OTHER:

2.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
 ☐ (b) Human tissues
 ☒ (c) Neither
- ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided)

We have developed a number of monoclonal antibodies to mouse T lymphocyte cell surface antigens which play critical roles in the process of T cell activation. One group of these reagents is directed to the receptor for interleukin-2 (IL-2) on activated murine lymphocytes. One of these antibodies, 7D4, detects an epitope on the IL-2 receptor distal to the ligand binding site, while a second antibody, 3C7, specifically inhibits the binding of radiolabeled IL-2 and is reactive with an epitope near the IL-2 binding site. As 3C7 and 7D4 act synergistically to inhibit IL-2 driven proliferation, a mixture of the two antibodies has been used to demonstrate that IL-2 plays a major role in all T cell proliferative responses in vitro and is very likely a universal growth hormone for T cells. The anti-IL-2 receptor antibodies have also been used to demonstrate the role of non-T accessory cells in the induction of IL-2 receptor expression on certain T cell subsets and to demonstrate that IL-2 may play an important role as a late acting B cell differentiation factor. The immunosuppressive agent, cyclosporin A, was shown to exert some of its immunological effects by inhibiting the maturation of receptor expression on activated T lymphocytes. Two other murine T cell differentiation antigens have also been studied with the aid of newly developed monoclonal antibodies. Thy-1 was shown to play a role in the activation process perhaps by functioning as a transducing molecule. L3T4, a marker for a subpopulation of T cells, was also shown to play a role in transmitting the activation signal. Monoclonal antibodies to all of these antigens should be attractive candidates for in vivo therapeutic use in attempts to selectively modulate or abrogate an ongoing immune response.

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| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00225-03 LI |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Effects of UV Light on Auto-Immune Disease in Auto-Immune Strains of Mice | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) | | |
| PI: I. Green | Senior Investigator | LI, NIAID |
| Others: J. Ansel | Guest Worker | LI, NIAID |
| | | |
| COOPERATING UNITS (if any) Arthritis Branch, NIADDKD (A. Steinberg and J. Mountz). | | |
| LAB/BRANCH Laboratory of Immunology | | |
| SECTION | | |
| INSTITUTE AND LOCATION National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20205 | | |
| TOTAL MAN-YEARS: <div style="text-align: center;">0.6</div> | PROFESSIONAL: <div style="text-align: center;">0.3</div> | OTHER: <div style="text-align: center;">0.3</div> |
| CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div> | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The purpose of this study is to examine the effects of UV light on auto-immune strains of mice. Humans with systemic lupus erythematosus often have worsening of disease after exposure to sun light. Three strains of auto-immune mice, BXSB, (NZBxBZW)_{F1} and MRL mice were exposed to either acute or chronic doses of UV light. It was observed that male BXSB mice had increased mortality after such treatment. They also had increases in levels of antibodies to ssDNA, increased antibodies to the TNP hapten and increased TNP-plaque forming cells in their spleen. Thus their B cells appeared activated. Sections of kidneys of male BXSB mice demonstrated increased inflammatory cells in their glomeruli. BXSB mice appear particularly susceptible to the deleterious effects of UV light. Our studies give a partial explanation for the deleterious effects of UV light in human SLE. </p> | | |

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| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00226-03 LI |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Rabbit Allotypes: Structure, Organization and Regulated Expression of Ig Genes | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) PI: R. G. Mage Senior Investigator LI, NIAID Others: E. Lamoyi Visiting Associate LI, NIAID N. McCartney-Francis Guest Worker LI, NIAID K. Bernstein Medical Staff Fellow LI, NIAID | | |
| COOPERATING UNITS (if any) LCMB, NCI (E. P. Reddy) and University of California, San Diego (S. Hedrick). | | |
| LAB/BRANCH Laboratory of Immunology | | |
| SECTION | | |
| INSTITUTE AND LOCATION National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20205 | | |
| TOTAL MAN-YEARS <div style="text-align: center; font-size: 1.2em;">3.0</div> | PROFESSIONAL: <div style="text-align: center; font-size: 1.2em;">1.5</div> | OTHER: <div style="text-align: center; font-size: 1.2em;">1.5</div> |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Our aims are to define the structure, evolution and organization of rabbit immunoglobulin genes and the analogous gene families that encode antigen-specific receptors of rabbit T cells. We seek to understand the mechanisms that regulate gene expression during lymphoid cell differentiation.</p> <p>Our comparisons of Basilea, b4,b5 and b9 cDNA sequences allowed us to construct an evolutionary tree in which the primordial Lagomorph kappa gene had a bas-like (K2) sequence. Structural genes encoding the K1 and K2 constant region isotypes have been identified on Southern blots of genomic DNAs of b5, b9 and bas rabbits. Deletions that occur in the intervening DNA sequence between the J cluster and C_K in "silent" K2 genes of b4 rabbits appear to also occur in the DNA of bas rabbits that express the K2 gene, although small differences could not be detected by our technique.</p> <p>An important and unanticipated level at which the expression and function of rabbit κ chains may be affected is the encoded protein itself. The locations and total numbers of cysteines affect the light chains' conformation and expression and may have a similar effect on analogous T cell receptor structures. Labeled cDNA encoding the putative β chain of the mouse T cell receptor, cross-hybridized to rabbit thymus mRNA. cDNAs prepared from this mRNA and clones of genomic DNA that also contain cross-hybridizing sequences are currently under study.</p> <p>A short restriction enzyme fragment from the first framework region of a VHa2 cDNA clone specifically hybridized to expressed mRNAs of VHa2 rabbits and not to those of VHa1 and VHa3 rabbits. About 10% of the phage plaques in a genomic library of VHa3 DNA, that were positive with a long VH probe, were positive with the short probe. Two different 8 kb subclones, selected from two different positive phage, contained at least two VH genes. In contrast with mouse VH genes which are usually 8-14 kb apart, these rabbit VH genes are only about 3 kb apart. Studies that are still in progress thus far indicate that all rabbits probably do not have the same genomic content of structural genes for the complex VHa allotypes.</p> | | |

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00227-02 LI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemistry of Lymphocyte Activation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Myron J. Waxdal, Sr. Investigator, LI, NIAID

COOPERATING UNITS (if any)

Philip Noguchi Sr. Investigator, BB, DBB

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been terminated.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00228-02 LI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Membrane Associated Glyconjugates and Glycoconjugate Receptors of Lymphoid Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Myron J. Waxdal, Ph.D., Sr. Investigator, LI, NIAID

COOPERATING UNITS (if any)

Claudine Kieda Centre National de Recherche Scientifique, Orleans, France

Michel Monsigny Centre National de Recherche Scientifique, Orleans, France

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD

TOTAL MAN-YEARS

PROFESSIONAL

OTHER

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☒ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type Do not exceed the space provided.)

This project has been terminated.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00229-03 LI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on Lymphocyte Differentiation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: W. E. Paul Chief, Senior Investigator LI, NIAID

Others: D. Cohen Medical Staff Fellow LI, NIAID

COOPERATING UNITS (if any) Dept Med. Microbiology, Stanford University School of Medicine, Stanford, CA (M. Davis); Dept Biology, University of California-San Diego (S. Hedrick); and A+R, NIADKD (A. Steinberg).

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:

0.6

PROFESSIONAL:

0.4

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The goal of this project has been to enumerate, identify and characterize genes uniquely expressed in B and/or T lymphocytes, with particular emphasis on those expressed at specific stages of maturation, activation, or differentiation. Through the use of subtractive cDNA-mRNA hybridization, "cell-specific" cDNA probes and cDNA libraries have been obtained. Such probes and libraries have thus far allowed the identification, cloning, sequencing and understanding of genomic organization of the genes encoding 1) the -chain of the T cell receptor and 2) the A class II MHC molecule. Furthermore, this approach has allowed the identification of a family of X chromosomal genes (XLR genes) uniquely expressed in lymphocytes. Among B lymphoma lines, presence of XLR mRNA correlates with the stage of maturation. XLR genes are closely linked to the x-linked immunodeficiency (xid) gene of CBA/N mice; indeed xid may be a member of the XLR gene family.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00259-03 LI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Ia Molecules and Immune Responses Genes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: W. E. Paul Senior Investigator, Chief LI, NIAID

Others: R. N. Germain Senior Investigator LI, NIAID
M. Brown Guest Worker LI, NIAID

COOPERATING UNITS (if any)

Harvard School of Public Health, Boston, MA (L. H. Glimcher).

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

1.0

PROFESSIONAL

0.2

OTHER

0.8

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Ia molecules are class II major histocompatibility gene products which are co-recognized with antigen by helper T cells and by related L3T4⁺ T cells. These molecules are products of immune response genes and as such determine the capacity of cells from individual animals to present specific antigens to T lymphocytes. This has suggested that the structure of the highly polymorphic Ia molecules determines which antigens may be presented and which cannot be presented. To examine this in more detail, mutant Ia molecules of the I-A^k type have been prepared by mutagenesis and selection of cells from an antigen-presenting line. These mutants have lost the capacity to present certain antigens to T cell clones which co-recognize that antigen and wild-type I-A^k Ia molecules. Genomic clones of the β chain of one mutant have been obtained and the mutation shown to consist of a single base change leading to a glutamic acid-lysine change at position 67, in the β_1 domain. Thus a single amino acid substitution profoundly effects the capacity of a class II molecule to be co-recognized with specific antigen.

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| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00349-02 LI |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Structure and Function of Murine Class II MHC Genes and Gene Products | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) | | |
| PI: | R. N. Germain | Senior Investigator LI, NIAID |
| Others: | M. A. Norcross | Medical Staff Fellow LI, NIAID |
| | N. S. Braunstein | Medical Staff Fellow LI, NIAID |
| | D. M. Bentley | Guest Worker LI, NIAID |
| | M. A. Brown | Guest Worker LI, NIAID |
| | J. Tou | Research Chemist LI, NIAID |
| | K. Nickerson | Research Biologist LI, NIAID |
| COOPERATING UNITS (if any) LI, NIAID (D. H. Margulies); Harvard School of Public Health, Boston, MA (L. H. Glimcher); and LI, NIAID (W. E. Paul). | | |
| LAB/BRANCH Laboratory of Immunology | | |
| SECTION | | |
| INSTITUTE AND LOCATION National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20205 | | |
| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
| 3.6 | 2.5 | 1.1 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Class II (Ia) gene products play critical roles in a variety of T lymphocyte responses. They are the primary stimulating antigens in allogeneic and syngeneic mixed lymphocyte repsonses, they "restrict" recognition of foreign antigens by Lyt1⁺ (L3T4⁺) T lymphocytes, and they control the ability of animals to respond to T dependent antigens (immune response [Ir] gene function). A combination of immunological and molecular genetic approaches is being used to gain an understanding of the structural basis for this recognition of Ia by T lymphocytes. Towards this goal, genomic or cDNA clones of A^{B,d,k}, A^{B,d,k,bml2}, E^{k,d}, E^{d,k}, and E^{B,k} have been isolated, and where necessary, sequenced. A_β and A_α genes^B have been transfected into B lymphomas or L-cells, and Ia expressing transformants obtained. These have been used to stimulate a variety of T cell hybridomas and clones, establishing the importance of both A_α and A_β polymorphic regions in forming restriction elements. Exon-shuffling between allelic A_β genes localized both serologic and T cell recognition sites to the highly variable β₁ domain. Sequence analysis of EMS induced A_β mutants also localized a critical site of function to a small region of the β₁ domain near the recently sequenced bml2 mutation. Finally attempts to construct transfectants expressing "hybrid" I-A molecules revealed an unexpected restriction on α:β chain assembly which maps to the β₁ domain. These results have shown the validity of this approach in determining the critical structural features of class II molecules recognized by T lymphocytes, and the molecular basis of Ia chain assembly. </p> | | |

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|---|-----------------|--|-----------|-----------------|--------------|-----------|---------|-----------------|--------------|-----------|--|-----------------|--------------|-----------|--|----------------|---------|-----------|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00394-01 LI | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders) Molecular Genetic Analysis of Lymphocyte Function | | | | | | | | | | | | | | | | | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI:</td> <td style="width: 33%;">D. H. Margulies</td> <td style="width: 33%;">Investigator</td> <td style="width: 33%;">LI, NIAID</td> </tr> <tr> <td>Others:</td> <td>J. A. McCluskey</td> <td>Guest Worker</td> <td>LI, NIAID</td> </tr> <tr> <td></td> <td>L. M. Hillstrom</td> <td>Staff Fellow</td> <td>LI, NIAID</td> </tr> <tr> <td></td> <td>D. A. Sobieski</td> <td>Chemist</td> <td>LI, NIAID</td> </tr> </table> | | | PI: | D. H. Margulies | Investigator | LI, NIAID | Others: | J. A. McCluskey | Guest Worker | LI, NIAID | | L. M. Hillstrom | Staff Fellow | LI, NIAID | | D. A. Sobieski | Chemist | LI, NIAID |
| PI: | D. H. Margulies | Investigator | LI, NIAID | | | | | | | | | | | | | | | |
| Others: | J. A. McCluskey | Guest Worker | LI, NIAID | | | | | | | | | | | | | | | |
| | L. M. Hillstrom | Staff Fellow | LI, NIAID | | | | | | | | | | | | | | | |
| | D. A. Sobieski | Chemist | LI, NIAID | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) Department of Genetics, Harvard Medical School, Boston, MA (J. C. Seidman); and LI, NIAID (R. Germain). | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Immunology | | | | | | | | | | | | | | | | | | |
| SECTION | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20205 | | | | | | | | | | | | | | | | | | |
| TOTAL MAN-YEARS | PROFESSIONAL | OTHER | | | | | | | | | | | | | | | | |
| 2.3 | 1.5 | 0.8 | | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided) <p>As part of our continuing effort to understand both the evolution and the structure-function relationships of the major histocompatibility antigens of the mouse, we have been involved in a number of studies applying the methods of recombinant DNA technology to the genes that encode these cell surface proteins. In particular, we have focussed on: <u>A.</u> The identification of genes highly homologous to (and thus, possibly allelic to) the H-2L gene of the BALB/c mouse in other inbred mouse strains and in wild mice as well. We have demonstrated a similar gene in virtually all mouse DNA that we have examined, and have cloned one such example from the C57Bl/6 mouse. <u>B.</u> The analysis of the recombinational events that occur between fragments of H-2 genes when they are introduced into mouse L cells by the method of calcium phosphate precipitation. Even in the absence of selective pressure, these tissue culture cells are capable of generating active and unique H-2 genes from exposure to overlapping parts of H-2D and H-2L or vice versa. This provides a novel system for generating H-2 mutant gene products for analysis of their function. <u>C.</u> The development of methods for introducing mouse class II (I-A) genes into either functional B cell lymphoma lines, or into mouse L cells. Surprisingly, the fibroblastoid L cells are capable of assuming the function of antigen-presenting cells when they are converted to I-A positive cells by such transfection procedures. <u>D.</u> Generating in vitro recombinant chimeric class II/class I genes with the intent of analyzing 1) the requirements for cell surface expression of MHC gene products, and 2) the role of particular domains of the class II or class I molecules in T cell recognition. <u>E.</u> Developing a molecular biological approach to the cloning of genes linked to and/or encoding the Mls locus of the mouse, located on chromosome 1, which is the only known non-major histocompatibility complex locus controlling a primary T cell proliferative response.</p> | | | | | | | | | | | | | | | | | | |

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00400-01 LI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunoregulatory Disorders in Systemic Lupus Erythematosus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PL: I. Green Senior Investigator LI, NIAID

Others: H. Suzuki Visiting Fellow LI, NIAID

COOPERATING UNITS (if any)

Arthritis Branch, NIADDK (A. Steinberg)

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:

1.6

PROFESSIONAL:

1.3

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Patients with systemic lupus erythematosus (SLE) have autoantibodies to T cells; in this study we looked, in particular, for antibodies to the interleukin-2 (IL-2)-receptor on T cells. Four out of ten sera from patients with SLE blocked the IL-2 induced proliferation of PHA blasts or IL-2 dependent T cells. However, no inhibition by SLE sera of binding of radiolabeled IL-2 monoclonal anti-IL-2 receptor antibody of ^{125}I - to these cells was observed. In a related study we examined whether B cells from patients with SLE were normally activated to proliferate in response to anti IgM. B cells from patients with SLE either responded poorly to such a stimulus or responded in a super-normal fashion. These responses were not correlated with the clinical state of the patient. In other experiments PHA supernatants containing stimulating lymphokines were added to the system. This had no effect on the response of the B cells from SLE patients that had been previously unresponsive.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00403-01 LI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Molecular Genetic Analysis of T Cell Receptor Structure and Repertoire

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R. N. Germain Senior Investigator LI, NIAID

Others: J. Miller Guest Worker LI, NIAID
R. Lechler Guest Worker LI, NIAID
J. Tou Research Chemist LI, NIAID

COOPERATING UNITS (if any)

University of California, San Diego, CA (S. Hedrick); Stanford University School of Medicine, Stanford, CA (M. Davis); and Dept of Pathology, Harvard Medical School, Boston, MA (M. Dorf).

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

1.0

PROFESSIONAL

0.5

OTHER

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Clonally distributed receptors on T lymphocytes provide the structural basis for selective antigen-specific regulatory and effector activities of these cells. To understand which receptors are expressed by the various T cell subsets showing distinctive antigen recognition spectra (eg class I restricted-CTL, class II restricted-helper cells, unrestricted-suppressor inducers), to determine the germ line repertoire from which these choices are made and the mechanisms controlling such choices throughout ontogeny, and to determine which structural features of these molecules account for their unique recognition and effector properties, it is necessary to clone representative members of this gene family. Such cloned genes can be sequenced, and also used as probes of germ line and expressed sets of receptor genes in different T cell subsets. Transfection of these cloned genes can be used as a tool to examine assembly of T cell receptor chains and to map the portions of the receptor involved in nominal antigen, restriction element, and alloantigen recognition. This approach can also establish whether so-called "T cell Ia" consists of epitopes on the receptor, and whether a modified form of the receptor constitutes a biologically active regulatory molecule (helper or suppressor factor). Towards these goals, the involvement of the recently identified T cell receptor gene locus in suppressor cell receptor formation has been examined.

LABORATORY OF IMMUNOREGULATION
1984 Annual Report
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Summary Report
Laboratory of Immunoregulation
October 1, 1983 through September 30, 1984

Anthony S. Fauci, M.D.
Chief, Laboratory of Immunoregulation, NIAID
Deputy Clinical Director, NIAID

Studies of the Human B Cell Cycle: Activation, Proliferation, and
Differentiation of Human B Lymphocytes in Normal and Disease States

Over the past year (1983-1984), we have utilized a model system previously developed in this laboratory for dissecting out the distinct phases of the human B cell cycle, i.e., activation, proliferation, and differentiation. We obtained suspensions of highly purified small resting (G_0 phase) B cells by countercurrent centrifugation elutriation. These G_0 cells could be induced to enter the cell cycle up to, but not beyond, G_1 whereby they enlarge, synthesize RNA, and express receptors for B cell growth factor I (BCGF-I). Upon exposure to BCGF-I, these cells enter S phase and undergo cycling; cycling B cells as well as in vivo-activated large B cells selectively respond by enhanced proliferation to a distinct BCGF (BCGF-II). These activated S phase cells then express receptors for B cell differentiation factor (BCDF) to which they respond by differentiation and Ig secretion. Other cytokines such as interleukin-1 (IL-1), and gamma interferon did not directly activate resting B cells but did synergize with BCGF in the induction of proliferation. Within the framework of this system of B cell cycle analysis, we have successfully identified and isolated functional subsets of human B cells on the basis of their state of activation by employing cell sizing techniques; the sequential expression of surface activation antigens recognized by monoclonal antibodies (4F2 and 5E9), which were developed in this laboratory; the synthesis of RNA and DNA; and the selective response to growth and differentiation factors. This constitutes the first successful identification and isolation of functional subsets of B cells in the human system.

Having delineated the role of various BCGFs, BCDFs, IL-1, and gamma interferon in human B cell function, we determined if factors such as IL-2, which classically act on T cells, could also influence B cell function. Using the anti-Tac monoclonal antibody which recognizes the IL-2 receptor on activated T cells, we demonstrated the expression of IL-2 receptors on normal activated B cells as well as established human B cell lines, whereas freshly separated (presumably resting) normal B cells did not express the Tac antigen. Immunoprecipitation analysis revealed that antigens of similar size with a molecular weight of 50-60 Kd can be precipitated with anti-Tac antibody from phytohemagglutinin (PHA)-stimulated normal T cell blasts and normal activated B cells as well as a cloned B cell line. Binding assays of IL-2 on the cloned B cell (HS1) revealed that B cells have significantly fewer sites and much less affinity of IL-2 receptors compared to PHA-stimulated normal T cell blasts. Finally, biological properties of the IL-2 receptor on B cells were examined by incubating B cells with recombinant IL-2. It was found that high concentrations of IL-2 induce significant enhancement of differentiation in Staphylococcus aureus Cowan I (SAC)-activated normal B cells. These results suggest that normal B cells may express functional IL-2 receptors or closely

related proteins, and IL-2 may play a significant role in the modulation of B cell function.

In anticipation of studies aimed at delineating the nature of the receptors for BCGF and BCDF on human B cells, as well as to facilitate the ultimate cloning of the genes for the production of factors, we successfully purified to homogeneity and characterized various BCGFs and BCDFs derived from panels of cloned and hybridized lines. In addition, we have performed partial amino acid sequencing of these factors and have produced monoclonal antibodies against them.

Finally, we have described, for the first time, the production of BCGF and BCDF from a transformed human B cell line and have purified to homogeneity the B cell-derived BCGF. Hence, this finding establishes that B cell growth is not totally dependent on T cell-derived BCGF, and the production of growth factor by B cell tumor lines may contribute to their ability to grow autonomously and may reflect an important component of the neoplastic potential of the cell. Furthermore, this raises the possibility that normal B cells may also produce BCGF for autotutilization. (Muraguchi, Kehrl, Ambrus, Le thi Bich-Thuy, Lane, Fauci, LIR/NIAID; Longo, MB/NCI; Butler, University of Alabama)

Immunoregulation of Antigen-Specific Human B Cell Responses

In 1980 we developed the first soluble antigen-induced, antigen-specific system for the production and assay of specific antibody in man. From 1980 through 1983, we precisely delineated the cellular and humoral components of the complex immunoregulatory events operative in this system. In 1983-1984, due to our use of lymphokines such as IL-2 and gamma interferon in clinical trials of patients with the acquired immunodeficiency syndrome (AIDS), we determined the in vitro effects of various lymphokines on this well-characterized antigen-specific system of antibody production. We demonstrated that IL-2 was a potent proliferative signal to peripheral blood lymphocytes in the absence of an exogenous activation signal and that it induced the secretion of antigen-specific immunoglobulin (Ig) from the otherwise unstimulated peripheral blood lymphocytes of individuals immunized with keyhole limpet hemocyanin. These potent direct effects of IL-2 in the absence of other required signals on specific and nonspecific immune function have important implications in the feasibility of its use as an immunomodulatory agent.

In 1982-1983 we delineated the monocyte requirements for the induction of antigen-specific responses at the T cell and B cell level. Over the past year (1983-1984), we further studied the role of HLA-DR encoded molecules in the presentation of antigen to human lymphocytes. We demonstrated a differential role of DR antigens on monocytes in antigen-stimulated versus mitogen-stimulated human lymphocyte responses whereby monocyte DR antigens were essential for the former but not the latter, and this did not appear to be related to the ability of monocytes to secrete IL-1. We further demonstrated that DR or Ia-bearing activated T cells were capable of presenting alloantigens but not soluble antigens to other T cells suggesting the inability of these activated T cells to process antigen and thus providing further insights into the complexities of antigen presentation. Finally, a number of transformed and nontransformed monocytoïd tumor cell lines and B

cell lines were employed to further study the genetic restrictions in antigen presentation. It was demonstrated that the existing serologically defined DR specificities are in fact broad classifications and encompass multiple molecular DR subtypes within a given specificity.

The first in vitro system for the study of specific antibody production to hepatitis B surface antigen by B cells from immunized individuals was developed, and the cellular requirements and interactions of the human immune response following booster immunization with hepatitis B vaccine were defined. The ability to evaluate antigen-induced, antigen-specific responses to hepatitis B surface antigen will be useful in evaluating the unique interaction between the human immune response and this clinically important viral infection.

Study of the antigen-specific B cell responses were facilitated by the development of cloned populations of antigen-specific T cells which exerted immunoregulatory effects on autologous or major histocompatibility complex (MHC)-compatible B cells (see below). (Le thi Bich-Thuy, Lane, Gerrard, Volkman, Fauci, LIR/NIAID; Cupps, Georgetown University)

Use of Hybridized and Cloned Populations of Immunocompetent Cells in the Study of Human Immune Function

In 1982-1983 we developed the first human T--T cell hybridoma from human peripheral blood which secreted BCGF in the absence of IL-2, BCDF, alpha interferon, or gamma interferon, thus allowing us to dissect out precisely the role of BCGF in the human B cell cycle. Over the past year (1983-1984), we have extended these studies and have developed a hybrid which secretes 2 separate molecules: a BCGF with a molecular weight of 18 Kd and a BCDF with a molecular weight of 32 Kd. These hybrids are not only an important source of monoclonal growth and differentiation factors but also reflect at the clonal level the immunoregulatory potential of the normal human peripheral blood T cell repertoire. We have also developed human T--T hybrids which secrete a factor(s) which suppresses Ig production by B cells in a pokeweed mitogen-driven system. The factor likely exerts its suppressive effect by selectively enhancing T8 proliferation while suppressing T4 proliferation.

Since the development by the LIR in 1980-1981 of the first panel of human T cell clones from peripheral blood which were reactive with soluble antigens to which the host had been immunized, we have extended these studies and have developed panels of antigen-specific T cell clones which grow independently of exogenous factors and require only the antigen in question and histocompatible antigen-presenting cells for growth. The ability of these clones to grow in the absence of exogenous factors has allowed us to assay for the elaboration of soluble factors following antigen stimulation of the clones. In this regard, these individual clones secrete, upon antigen stimulation, multiple lymphokines including IL-2, BCGF, BCDF, and interferon.

The first parasite-specific human T cell clones were developed from the peripheral blood of an individual infected with loa loa. These clones elaborated a soluble factor which directly stimulated IgE production in both autologous and allogeneic B cells; this IgE stimulation was suppressed by T cells. Thus, the mechanism for hyper-IgE observed in certain parasitic diseases may reflect the response to a specific factor produced in response to

a parasitic antigen, and its down regulation may also be controlled by T cells.

In studies aimed at delineating the role of human T-cell leukemia virus (HTLV) infection in immune dysfunction, we infected an antigen-specific T cell clone with HTLV-I. Infection with HTLV converted the clone from a truly antigen-specific, MHC-restricted inducer of B cell function to a line which spontaneously and indiscriminantly induced polyclonal Ig production in an MHC non-restricted manner. This mechanism might explain the apparently paradoxical finding in AIDS of severe immunosuppression associated with polyclonal activation of certain lymphocyte subsets resulting from infection of T cells with HTLV-III. In this regard, clonal studies of T cells from patients with AIDS demonstrated that these patients have markedly reduced cloning efficiencies of both T4 (helper/inducer) and T8 (cytotoxic/suppressor) subsets. However, the subsets that were in fact clonable functioned normally. Furthermore, we developed a panel of nonspecific T cell clones from normal human peripheral blood by limiting dilution technique and correlated the phenotypic characteristics of the clones with their functional capabilities in modulating polyclonal B cell activity.

B cell lines from individuals with inherited disorders of phagocytosis (Chediak-Higashi syndrome and chronic granulomatous disease) were developed and characterized, thus making available potentially unlimited numbers of cloned cells for biochemical and molecular study. Established B cell lines were studied for their capacity to respond to various BCGFs and BPDFs. These cell lines served as models for dissecting the mechanisms of B cell responses to exogenous factors. In addition, B cell lines were identified which autonomously produced their own growth factors. Finally, we developed a stable fusion partner (a pre-B cell line which was made hypoxanthine/aminopterin/thymidine sensitive) for use in human-human B cell hybridizations. This line is now resulting in excellent fusions with normal activated B cells (looking for a fusion with a high density of expressed BCGF receptors) and with an HTLV-infected B cell line (looking for a fusion with a high density of expressed Tac receptor). (Ambrus, Volkman, Goldstein, Fauci, LIR/NIAID; Nutman, Ottesen, LPD/NIAID; Popovic, Gallo, LTCB/NCI; Buescher, Gallin, LCI/NIAID; Butler, University of Alabama)

Pharmacologic Modulation of the Human Immune Response

Over the past 12 years, the LIR has been involved in studies aimed at delineating the mechanisms whereby immunosuppressive agents such as corticosteroids (CS) and cytotoxic drugs, which are used in the treatment of inflammatory and/or immune-mediated diseases, modulate the human immune response. Over the past year, we have examined the differential effects of in vitro CS on various phases of the B cell cycle, experiments made possible by the development of our currently employed model system for examining distinct phases of the activation, proliferation, and differentiation of resting human B cells. We demonstrated that CS exert a selective inhibitory effect on early events in the B cell cycle which subsequently lead to inhibition of total RNA and DNA synthesis. In contrast, the proliferative responses of in vitro or in vivo preactivated B cells are relatively refractory to the suppressive effects of CS as is the differentiation of preactivated B cells to the Ig-secreting state.

Cyclosporin A (CsA) is generally thought of as an immunosuppressive agent which selectively exerts its effects on helper T cells. However, using our model of B cell cycle analysis, we demonstrated that CsA had a selective inhibitory effect on the activation phase versus the proliferative phase of the B cell cycle, which is examined following preactivation. Thus, CsA does not have significant effects on B cell function, and similar to CS, it selectively suppresses an early step of human B cell activation and has little inhibitory effect of the subsequent factor-dependent proliferation and differentiation phases of the cell cycle. These studies add further insight into the potential for pharmacologic modulation of selective components of the human immune response. (Bowen, Muraguchi, Fauci, LIR/NIAID; Cupps, Georgetown University)

Studies in the Acquired Immunodeficiency Syndrome (AIDS)

Over the past year, the LIR has been intensely involved in the study of AIDS. Our work has centered on four main areas: description of the epidemiologic, clinical, pathologic, and pathophysiologic manifestations of AIDS and the pre-AIDS syndromes; characterization of the immunologic dysfunction in AIDS; delineation of the causative agent(s) of the disease; and the development of therapeutic strategies for both the complications and the underlying immunologic defect of AIDS.

We have demonstrated that individuals in risk groups for AIDS, patients with pre-AIDS syndromes, and patients with definite AIDS create a spectrum of immunologic dysfunction with those patients who have developed opportunistic infections being the most severely immunocompromised of the group. Of all the standard screening tests for immunologic function examined in these groups, the ability to respond to soluble antigen appeared to be the most sensitive for detecting a critical defect in immunologic function. Thus, the immunologic evaluation of these patients parallels their clinical course.

The LIR was among the groups which provided numerous clinical specimens to a variety of virologists at the NIH in the effort to isolate the etiologic agent of AIDS. At the present time, it appears that a human T cell tropic retrovirus (HTLV-III/LAV) is the unifying causative agent of AIDS. Studies attempting to delineate what, if any, concomitant host factors are required for the development of AIDS following exposure to this virus are currently underway. In addition, a series of experiments are being carried out which are directed towards describing the effect(s) of this virus on normal lymphocytes. At the present time, it appears that this virus is particularly cytotoxic and cytopathic for the T4 lymphocyte.

We have demonstrated that the immune systems of patients with AIDS are characterized by both quantitative and qualitative defects in cell-mediated immunity. Quantitatively, these patients exhibit reduced numbers of helper/inducer T lymphocytes and elevated, normal, or decreased numbers of suppressor/cytotoxic T lymphocytes. Qualitatively, the suppressor/cytotoxic lymphocytes appear to function normally when provided with an intact inducer signal either in the form of normal allogeneic helper/inducer T lymphocytes or lymphokines, while the helper/inducer subset has been found to exhibit intact responsiveness to mitogens with a selective defect in the ability to respond to soluble antigen. This selective quantitative and qualitative defect in the helper/inducer T lymphocyte subset was one of the earliest observations

supporting the hypothesis that this disease is due to infection with a T4-tropic retrovirus similar to the viruses which have been associated with forms of human T cell leukemia/lymphoma. Additional studies of B cell function in these patients have revealed a decreased responsiveness to a panel of in vitro activation signals and an enhanced responsiveness to BCGF.

There are no reported cures or reversals of this illness. Accordingly, the LIR has launched a major effort in the area of the therapy of AIDS. Protocols are currently being carried out which study alternate therapeutic approaches to the treatment of two of the most common opportunistic infections in this group, Pneumocystis carinii and Mycobacterium avium-intracellulare. In collaboration with the National Cancer Institute, we are comparing the effects of chemotherapy and alpha interferon in the treatment of patients with Kaposi's sarcoma. Correction of the immunologic defect is being attempted in three separate clinical trials: a phase I trial of interleukin-2, a phase I trial of immune (gamma) interferon, and attempted immunologic reconstitution via lymphocyte transfers. These studies, have proven to be extremely informative concerning the pharmacokinetics of these new recombinant clinical agents and their ability to modulate the human immune system. While they have been of little clinical value thus far in the treatment of patients with AIDS, the information gained should be of great value in the future use of these compounds in immunologically mediated diseases. (Lane, Margolick, Fauci, LIR/NIAID; Masur, CCM/CC; Gelmann, MB/NCI; Gallo, LTCB/NCI).

Studies in the Idiopathic Hypereosinophilic Syndrome (HES)

Over the past several years, we have been involved in studying the clinical and pathophysiologic aspects of idiopathic HES. Most recently, we have examined the nature of the intracytoplasmic granules of the eosinophil and whether their contents may be involved in mediating the tissue damage in this disease. Over the past year, we have characterized the distinct components of the eosinophil cytoplasmic granules and have attempted to correlate these with pathophysiologic manifestations of disease. We have identified and purified to homogeneity several distinct proteins which are contained in the human eosinophil granules. These include the major basic protein, the eosinophil cationic protein, and the eosinophil-derived neurotoxin. Each of these distinct proteins has been implicated in the in vitro-demonstrated mechanisms of one or more in vivo pathophysiologic findings in HES. Finally, degranulation of eosinophils from HES patients by the calcium ionophore A23187 was studied. It was demonstrated that indeed eosinophils are potent secretory cells and their elaboration of leukotrienes and other arachidonic acid lipoxygenase products may help mediate inflammation in allergic diseases including asthma, parasitic infections, and HES. (Fauci, LIR/NIAID; Gleich, Mayo Clinic; Henderson, University of Washington)

Studies in Graves' Disease

It is strongly suspected that the lymphoid cells which infiltrate the thyroid glands of patients with Graves' disease are directly or indirectly responsible for the tissue damage in the gland. Thus, we phenotypically characterized the T lymphocyte subsets infiltrating the thyroid glands of patients with Graves' disease and demonstrated that unlike glands from normals, the follicular epithelium was infiltrated with a population of

mononuclear cells comprised almost entirely of T cells with the T8 suppressor phenotype. We further studied the functional capabilities of T cells from Graves' disease patients at the clonal level. Although we failed to isolate anti-thyroid antigen-specific T cell clones, we did isolate T8+ clones which produced large amounts of BCGF. The presence of increased numbers of T8 cells in the glands of these patients which are capable of producing BCGF suggest that they may play an important role in the aberrant B cell response in Graves' disease. (Margolick, Fauci, LIR/NIAID; Hsu, LP/NCI; Burman, Walter Reed Army Medical Center)

Studies in Autoimmune and Other Immune-Mediated Diseases

Over the past year, we have continued studies aimed at delineating the nature of the B cell hyperactivity in Sjögren's syndrome (SS). Since SS presents a wide spectrum of disease manifestations ranging from benign to malignant B cell lymphoproliferation, we investigated whether a monoclonal B cell process was in effect at the early stages of disease activity. We found that two-thirds of patients with SS without a lymphoproliferative disease had free monoclonal lambda light chains in their sera. These findings lend credence to the concept that the monoclonal process in SS begins early in the disease process in a subset of B cells.

Since patients with lymphomatoid granulomatosis and certain types of lymphomas may develop granulomatous reactions as well as hemophagocytic syndromes, we investigated whether factors secreted by the malignant T cells might be responsible for the induction of these phenomena. We demonstrated a factor in T cell culture supernatants from several such patients which augmented the phagocytosis of IgG-coated ox red blood cells by the human monocyte/macrophage line U937. Its stimulation of phagocytosis was independent of an increase in number of Fc receptors and was not gamma interferon. This factor may play a pathogenic role in the hemophagocytic syndromes associated with certain T cell malignancies and immunodeficient states. (Lane, Fauci, LIR/NIAID; Papadopoulos, CPD/CC; Moutsopoulos, University of Ioannina, Greece; Jaffe, Simrell, Cossman, LP/NCI)

Clinical, Immunologic, Pathogenic, and Therapeutic Studies in the Spectrum of Vasculitis

Over the past 16 years, we have been prospectively studying the largest group of patients with the vasculitic syndromes of any center in the world. Over the past year, these studies have continued and accelerated. Clinical, pathophysiologic, immunopathogenic, and therapeutic results have allowed us to design a revised categorization scheme for the vasculitides which has reached worldwide acceptance. Sixteen years ago, we designed and implemented an aggressive chemotherapeutic regimen consisting of chronic low-dose CY together with alternate-day CS for treatment of several of these formerly universally fatal diseases such as Wegener's granulomatosis and systemic vasculitis of the polyarteritis nodosa group. Using this regimen, we have affected long-term remissions and cures in greater than 90% of patients. This landmark study has now led to this regimen being successfully used throughout the world and has completely changed the previously grave prognosis of these diseases to one of almost invariable remission. We have extended these studies and now are treating a number of other vasculitic syndromes such as isolated central

nervous system vasculitis, Takayasu's arteritis, and the systemic vasculitis of the connective tissue diseases with similar success.

Furthermore, these patient populations have been utilized to precisely delineate aberrations of activation and immunoregulation of lymphoid cell function in man and have served as excellent models for the concomitant study of the normal human immune system. In addition, the precise effects of various therapeutic regimens, particularly CS and cytotoxic agents, on human lymphoid cell activation and immunoregulation have been described. (Fauci, Volkman, Lane, Leavitt, LIR/NIAID; Cupps, Georgetown University).

Future Plans and Objectives

Future plans and directions for studies of the B cell cycle are aimed at a more precise delineation of the signals required for the induction of resting B cells into the cell cycle through activation, proliferation, and differentiation. Membrane events will be examined and the role of phosphokinase C in the events following crosslinking of membrane Ig by anti-Ig reagents will be studied. We have previously demonstrated that upon activation, human B cells express acceptor sites for BCGF through which they respond to the presence of BCGF by cycling. BCGF can be absorbed out from factor-containing supernatants by activated B cells which express these receptors. Furthermore, we have demonstrated that certain B cell lines and activated B cell subsets express receptors for BCDF through which they respond to the presence of BCDF by differentiation and Ig secretion. In similar fashion, BCDF can be absorbed out by these receptor-bearing cells. Over the next year, we will place a major effort towards identifying, purifying, and characterizing the BCGF and BCDF receptors on activated B cells. We are currently purifying to homogeneity BCGF and BCDF which we have obtained from various T and B cell lines. We will radiolabel these highly purified factors for the purpose of performing binding studies directed at immunoprecipitating the cellular receptors. In addition, monoclonal antibodies are being produced against these factors and studies are underway to determine the active moiety in the molecule responsible for factor activity. Since we have at our disposal cloned lines which are producing these factors and since we are currently preparing highly purified factor, we plan to place a major effort into cloning the genes for the expression of these factors.

We have already identified and characterized two distinct BCGFs and at least one BCDF, as well as a factor which directly activates resting B cells. These factors are all derived from hybridized and/or cloned lines. It is highly likely that there are several more factors which act directly on B cells at various phases of the B cell cycle. We plan to continue our studies aimed at identifying and isolating the spectrum of soluble factors involved in the immunoregulation of human B cell responses.

We will continue our studies on the clonal analysis of human T cell subsets in order to more precisely define the complex interactions involved in the regulation of human B cell function by these diverse populations of cells. This will be done with antigen-specific clones as well as nonspecific T cell populations which reflect the broad scope of the available T cell repertoire. In addition to the study of the specific and nonspecific functional capabilities of these cells, they will be used as sources of monoclonal lymphokines which will allow even more precise definition of their role in the regulation of the B cell cycle. Furthermore, T cell clones obtained from patients with lymphoproliferative diseases as well as non-neoplastic diseases characterized by immunoregulatory abnormalities will be assayed for the secretion of factors which exhibit either normal or aberrant immunoregulatory activity. In addition, we will assay for factors which are responsible for abnormal cellular activation and/or proliferation such as hemophagocytosis by macrophages in certain lymphoproliferative diseases or eosinophilic proliferation in the idiopathic HES.

Our major commitment to the study of the pharmacologic modulation of the human immune response will continue. We will investigate the effects of

immunosuppressive agents such as CS, CY, and CsA on the various distinct phases of the B cell cycle. Also, given the availability of model systems for factor production, receptor expression, and factor binding to cellular receptors, we will explore the specific effects of these immunosuppressive agents on these phenomena. Furthermore, we will initiate further studies on the effects of various lymphokines such as IL-2 and gamma interferon in antigen-specific as well as nonspecific responses of human B and T cells. These studies will have important implications given the fact that these immunomodulatory agents are already in use therapeutically in phase I clinical trials in diseases such as AIDS, which is characterized by severe immune dysfunction.

Studies on the immunopathogenic mechanisms of certain immune-mediated diseases will continue. Patients with a variety of diseases characterized by aberrancies of immune function will continue to serve as a source of material for studies aimed at delineating the mechanisms of immune dysfunction. Since we have now established our model of B cell activation, we will direct studies at more precisely defining the defects of B cell activation and/or immunoregulation in certain of these diseases by integrating our studies into the framework of the discrete steps of B cell activation, proliferation, differentiation, and immunoregulation.

Over the next year, a major effort will be undertaken to apply the molecular biological approach to the study of the immunoregulation of human B cell responses. We will attempt to clone the genes for BCGF and BCDF; we will attempt to develop molecule probes for the induction of expression of receptors for the various growth and differentiation factors. We will study the gene rearrangements associated with the B cell activation process and will examine the relationship between activation of the myc gene and the expression of B cell activation.

Over the next year, an intense effort will be directed towards delineating the role of HTLV-III in modulating the human immune response. In this regard, lymphocyte subpopulations from patients with AIDS will be examined for evidence of viral transformation through the techniques of cocultivation and DNA hybridization. These studies should reveal the precise cellular target of natural infection with HTLV-III. Additionally, lymphocyte subpopulations from healthy control subjects will be infected with this virus and the subsequent changes in immunocompetency measured. The role of cofactors in allowing HTLV infection to occur will be studied. The potential for blockage of in vitro infection with pharmacologic agents will be investigated, and any promising leads for therapy extended to clinical trials. Now that a serologic test for antibodies to HTLV-III is available, a large scale study is planned to determine the prevalence of antibodies to this retrovirus in three groups of individuals: sexual contacts of known AIDS patients (both homosexual and heterosexual), non-sexual contacts living in households with AIDS patients, and hospital personnel delivering care to AIDS patients. Antibody-positive individuals and appropriate antibody-negative controls will be more carefully examined for evidence of altered immune function and viral shedding of HTLV-III. A complimentary study will examine a wide variety of body fluids from patients with AIDS in an attempt to identify sites of virus excretion. In addition to the studies mentioned above, in the basic research arena, efforts will be directed towards elucidating the mechanisms of the B cell activation characteristic of AIDS. These studies

will sort out whether or not this B cell activation is due directly to the viral transformation of the B cell by a known virus such as Epstein-Barr virus, cytomegalovirus, or HTLV-III or secondarily to the spontaneous release of B cell activation factors by T lymphocytes. In the area of therapy, the LIR will continue its collaborative efforts with the Clinical Center and the National Cancer Institute in examining the role of a variety of therapies directed towards the neoplastic and infectious complications of AIDS, with a particular emphasis on how these therapies affect the immune systems of the recipients. Additionally, we will continue our own studies on the use of immunomodulators in AIDS, moving to combination therapy once sufficient single agent data are obtained. It is hoped that this sort of information, even if not directly applicable to AIDS, may be of value in the future use of these compounds in the treatment of other immunologically mediated diseases.

Administrative, Organization, and Other Changes

The Laboratory of Immunoregulation (LIR) was established in 1980 and is now four years old. The major theme of the LIR is to study the precise mechanisms of activation and immunoregulation of human immunocompetent cells, particularly B lymphocytes, in normal individuals and in a variety of disease states characterized by abnormalities of immune function. In addition, the LIR continues to conduct the majority of the clinical studies which are carried out in the NIAID intramural program within the Clinical Center.

According to the plan which was put forth at the time the LIR was established, gradual expansion has occurred predominantly by drawing from the existing Medical Staff Fellow pool individuals who would remain within the program for variable periods of time both to maintain the continuity of ongoing studies as well as to serve as a source of individuals who might potentially and ultimately assume senior positions in the laboratory. In addition, when appropriate and necessary, individuals would be recruited from outside the NIH as the program expands over the years. In this regard, Drs. H. Clifford Lane and David J. Volkman were designated Senior Investigators in 1983 and will continue in that position. Dr. John H. Kehrl, who completed his three-year Clinical Associateship in June 1983, has remained within the Public Health Service over the past year. Dr. Kehrl will remain within the LIR and will assume a position as Senior Investigator over the coming year. In the winter of 1983, Dr. Ulrich K. Siebenlist joined the LIR from Dr. Philip Leder's laboratory at the Harvard Medical School, Department of Medical Genetics. He has assumed the position of Visiting Associate but will be converted to the position of Senior Investigator as soon as is feasible. Dr. Siebenlist will provide the LIR with expertise in molecular biology. We are currently recruiting an additional technician to assist him. Drs. Randi Y. Leavitt and Joseph B. Margolick will have their three-year appointments extended for a fourth year as Medical Staff Fellows. Drs. Julian L. Ambrus and Debra L. Bowen will enter their third year as Medical Staff Fellows. As with previous individuals within this program, it is expected that at least one and hopefully both of them will be able to stay on an additional year. Dr. Harris Goldstein has completed his first year Medical Staff Fellowship and will continue within the program. In November 1983 Dr. Le thi Bich-Thuy joined the LIR from the laboratory of Professor Jean-Pierre Revillard at the Hopital Herriot in Lyon, France. She will remain at the LIR for at least an additional year and hopefully for a third year. In July 1984 Drs. Peter Bressler and Scott Koenig joined the intramural program as first-year Medical Staff Fellows. Drs. Shohken Tomita and Toshimasa Nakagawa will join the laboratory in spring and summer 1984 as Visiting Fellows. Dr. Tomita comes from the laboratory of Dr. Hamaoka in Osaka, Japan, and Dr. Nakagawa comes from the laboratory of Dr. Tadimitsu Kishimoto in Osaka. In July 1984 Dr. Alain Rook joined the laboratory to supply additional expertise in studying cytotoxic cell functional capabilities in patients with the acquired immunodeficiency syndrome. Over the past year, Dr. Marie M. Hippolyte-Deschamps from Port-au-Prince, Haiti, has been a Guest Researcher in the LIR. The purpose of her stay is to acquaint herself with the approach which the NIAID has been taking towards the clinical and investigative aspects of AIDS. Over the past year, we have added a nurse clinician, Mrs. Margaret Megill, to the staff for the purposes of helping with the intramural AIDS program. In addition, a student appointment was given to Mrs. Linda M.

Thompson for the purpose of helping with the AIDS program. In August 1984 Dr. Anthony Weetman from Wales will join the laboratory as a Guest Researcher.

Leaving the LIR this year will be Dr. Atsushi Muraguchi, who will return to the faculty of the University of Osaka. In addition, Drs. B. Lauren Charous and Theresa L. Gerrard will have completed their respective three- and four-year appointments in the laboratory. Ms. Lucy A. Renzi has completed her first year as secretary to the Laboratory Chief and has proved to be an extraordinarily important component of the laboratory's function. Ms. Ann C. London has completed her second year as Editorial Assistant and continues to provide invaluable editorial help to the members of the laboratory. Ms. Lori Schlaffer has joined the LIR as a Clerk Typist. Three new technicians have joined the laboratory to replace individuals whose slots were vacated. The new members of the technician staff include Ms. Julia H. Grove, Ms. Sandra E. Higgins, and Ms. Norma L. Witzel.

The laboratory space remains consolidated in the B Wing of the 11th floor of the Clinical Center. In this regard, Dr. Siebenlist has assumed the module 11B-05 for the initiation of his molecular biology unit.

Honors, Awards, and Scientific Recognition

Over the past year, members of the Laboratory of Immunoregulation predominantly in the person of Dr. Anthony S. Fauci, Chief, LIR, have received a number of awards and honors. First, Dr. Fauci has served on a number of committees of scientific note. He completed his final year as Program Chairman for the national meeting of the American Association of Immunologists. He is serving on the Committee on Clinical Immunology and Immunopathology of the American Association of Immunologists and represents the American Association of Immunologists on the Examinations Committee of the American Board of Allergy and Immunology. This past year he assumed his position on the Board of Directors of the American Board of Allergy and Immunology and is the co-director of the Diagnostic Laboratory Immunology Board. He continues as a member of the American Federation for Clinical Research President's Public Policy Advisory Committee and this year was made a member of the Scientific Advisory Board of the Medical Biology Institute of La Jolla, California, as well as a member of the External Advisory Committee of the Multipurpose Arthritis Center at Duke University Medical Center. He is also a member of the Honorary Advisory Board of the Italian-American Medical Association. In addition, he has been elected to membership in the Peripatetic Club. Over the past year he was a participant in the U.S.A.-Japan Immunology Advisory Conference held in Honolulu, Hawaii.

Dr. Fauci serves on a number of editorial boards of journals concerned with the areas of immunology, allergy, and infectious diseases. He remains Associate Editor in charge of allergy and immunology of the American Journal of Medicine. Over the past year he has completed his term on the Editorial Board of The Journal of Clinical Investigation as well as The Journal of Infectious Diseases. He currently maintains his position on the Editorial Boards of The Annals of Allergy, The Journal of Immunopharmacology, Clinics in Immunology and Allergy, EOS, Clinical and Experimental Rheumatology, La Ricerca, Clinical Immunology and Immunopathology, and the Physicians Journal Update. He also continues to co-edit, with Dr. John I. Gallin, the series which he and Dr. Gallin established three years ago entitled ADVANCES IN HOST DEFENSE MECHANISMS. After having edited the first edition of the highly successful book CURRENT THERAPY IN ALLERGY AND IMMUNOLOGY with Dr. Lawrence M. Lichtenstein, Dr. Fauci will undertake editing the second edition of that volume. Over the past year, Dr. Fauci was elected to the Advisory Board of the Journal of Clinical Immunology. He was made the Consulting Editor for North America of the journal Thymus. He was elected to the Editorial Board of the journals Immunopharmacology, the Journal of Molecular and Cellular Immunology, and Cellular Immunology. In addition, he was appointed as Associate Editor of the textbook entitled CURRENT THERAPY IN INTERNAL MEDICINE. Of note is the fact that over the past year Dr. Fauci has assumed the prestigious post as Editor of HARRISON'S PRINCIPLES OF INTERNAL MEDICINE. He will be responsible for editing the immunology, rheumatology, and allergy sections of that book as well as portions of the infectious disease and oncology sections. Finally, Dr. Fauci has contributed a number of invited chapters covering a variety of subjects for most of the major textbooks of medicine as well as subspecialty textbooks in immunology, allergy, and infectious diseases.

As part of the recognition for scientific accomplishments, clinical investigators may be asked to visit outside institutions and serve for periods

of from two to five days as Visiting Professors within a given institution. In this regard, Dr. Fauci has been asked and did serve as Visiting Professor at several major institutions throughout the year. Among these were two prestigious Visiting Professorships: The John S. Lawrence Visiting Professor of Medicine at the University of California, Los Angeles Medical Center, and the Irving H. and Martha L. Lepow Visiting Professorship at the University of Connecticut School of Medicine.

In addition, Dr. Fauci was asked to give several major or named lectureships during the year. He was an invited symposium speaker at the Fifth International Meeting of the International Society for Sexually Transmitted Diseases Research. He was an invited symposium speaker at the prestigious Stanhope Bayne-Jones Memorial Lectureship at the Johns Hopkins University School of Medicine. He was an invited symposium speaker at the Infectious Diseases Society of America National Meeting. In addition, he was an invited symposium speaker at the 17th Annual Congress of the American Association of Clinical Immunology and Allergy. He was an invited symposium speaker at The New York Academy of Sciences Conferences on the Acquired Immunodeficiency Syndrome. He delivered the prestigious John S. Lawrence Lecture at the University of California, Los Angeles Medical Center. He was an invited symposium speaker at the UCLA Symposium on the Acquired Immune Deficiency Syndrome. He was the annual lecturer at the Biochemistry Society of the University of Rochester Medical School. He delivered the Robert A. Cooke Memorial Lectureship to the American Academy of Allergy 40th Annual Meeting and was an invited symposium speaker at the Annual Meeting of the American Society for Microbiology, as well as the Annual Meeting of the American Association of pathologists. He delivered the Irving H. and Martha L. Lepow Lectureship at The University of Connecticut School of Medicine and was an invited panel discussion leader at the 65th Annual Session of the American College of Physicians. At this same time he was also an invited professor in the Meet the Professor Program. He delivered the 20th Annual Harold G. Pritzger Memorial Lectureship at the University of Toronto and the Academy of Medicine of Toronto. He was an invited Plenary Session Speaker at the American Federation for Clinical Research National Meeting in the program on Frontiers of Science. He delivered the prestigious Annual Morgagni Medical Society Lectureship of the New York Academy of Medicine and was the Chairman of the Minisymposia on Disorders of Immune Regulation in Man at the 66th Annual Meeting of the American Association of Immunologists. At that same minisymposium, Dr. H. Clifford Lane delivered an invited lecture. Finally, the work of the LIR and its members was widely recognized in that a large number of papers from the laboratory were chosen to be presented at the highly competitive programs of the American Association of Immunologists as well as the national meeting of the AAP/ASCI/AFCR.

Finally, over the past year Dr. Fauci has received several prestigious awards. These include the Squibb Award of the Infectious Diseases Society of America, the United States Public Health Service Distinguished Service Medal, and the Food and Drug Administration Commissioner's Special Citation, an award which he shared with Dr. Lane. Dr. Lane was also awarded the NIH Commendation Medal.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00210-04 LIR

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Immunoregulation of Human Lymphocyte Function in Normal and Disease States

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: A. S. Fauci Chief LIR, NIAID

| | | | |
|---------|---------------|----------------------|------------|
| Others: | H. C. Lane | Senior Investigator | LIR, NIAID |
| | D. J. Volkman | Senior Investigator | LIR, NIAID |
| | J. H. Kehrl | Clinical Associate | LIR, NIAID |
| | T. L. Gerrard | Staff Fellow | LIR, NIAID |
| | A. Muraguchi | Visiting Fellow | LIR, NIAID |
| | R. Y. Leavitt | Medical Staff Fellow | LIR, NIAID |

COOPERATING UNITS (if any)

Georgetown University, Washington, DC (T. R. Cupps).

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

8

PROFESSIONAL

6

OTHER

2

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

The human B cell cycle was analyzed with regard to the distinct phases of activation, proliferation, and differentiation. Resting B cells were isolated and driven through the cell cycle by a sequential series of signals. The distinct roles of the spectrum of growth and differentiation factors for human B cells were precisely delineated. These factors which were derived from human T--T cell hybridomas as well as cloned T cell lines were purified and characterized in preparation for amino acid sequencing and ultimate cloning of genes for their expression. For the first time in the human system, a transformed B cell line was identified which produced a B cell growth factor (BCGF). Production of BCGF by B cell tumor lines may contribute to their ability to grow autonomously and may reflect an important component of the neoplastic potential of B cells as well as the normal autotutilization of factors. IL-2 receptors were identified on normal activated human B cells which suggests a role for this factor in normal B cell function. The immunoregulation of antigen-specific human B cell responses was studied including the role of various Ia positive cells in antigen presentation and the effect of in vitro IL-2 on antigen-specific T cell clones on human B cell function. The first in vitro system for the study of specific antibody production to hepatitis B surface antigen was developed. The pharmacologic modulation of the distinct phases of the human B cell cycle was studied, and it was demonstrated that corticosteroids and cyclosporin A exhibited selective suppressive effects on the early events of B cell activation with relatively little effects on proliferation and differentiation. Finally, the selective effects of cyclophosphamide on certain distinct phases of the human B cell cycle were demonstrated.

| | | | |
|---------|-------------------|----------------------|------------|
| Others: | J. B. Margolick | Medical Staff Fellow | LIR, NIAID |
| | B. L. Charous | Medical Staff Fellow | LIR, NIAID |
| | D. L. Bowen | Medical Staff Fellow | LIR, NIAID |
| | J. L. Ambrus, Jr. | Medical Staff Fellow | LIR, NIAID |
| | Le thi Bich-Thuy | Guest Worker | LIR, NIAID |

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00211-04 LIR

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Study of Human Lymphocyte Subsets Employing Cloning and Hybridoma Technology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: A. S. Fauci Chief LIR, NIAID

Others: D. J. Volkman Senior Investigator LIR, NIAID
 H. C. Lane Senior Investigator LIR, NIAID
 J. L. Ambrus, Jr. Medical Staff Fellow LIR, NIAID
 J. B. Margolick Medical Staff Fellow LIR, NIAID
 H. Goldstein Medical Staff Fellow LIR, NIAID
 S. Tomita Visiting Fellow LIR, NIAID

COOPERATING UNITS (if any)

Laboratory of Parasitic Diseases, NIH (T. Nutman, E. Ottesen);
 Laboratory of Tumor Cell Biology, NIH (M. Popovic, R. C. Gallo); University of
 Alabama, Birmingham, AL (J. L. Butler).

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

3

PROFESSIONAL:

2

OTHER:

1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreserved type. Do not exceed the space provided.)

A number of human T--T hybridomas were developed which secreted a variety of immunoregulatory factors including B cell growth factor (BCGF), B cell differentiation factor (BCDF) and various suppressor factors. In addition, a number of T cell lines were identified and propagated which secreted similar BCGFs and BCDFs. These lines serve as excellent sources of virtually unlimited quantities of homogeneous factors which can be purified, amino acid sequenced, and ultimately used in studies aimed at cloning the genes for these factors. Further studies are directed at defining the nature of the growth factor receptors on the target cells in question. The original observation that certain human B cell lines secreted their own BCGF and/or BCDF was made and the factors were precisely characterized. We have also developed panels of soluble antigen-specific human T cell clones which grow independently of exogenous factors. Upon stimulation with the appropriate antigen, these antigen-specific clones secrete multiple lymphokines including IL-2, BCGF, BCDF, and interferon. We infected an antigen-specific T cell clone with human T cell leukemia/lymphoma virus (HTLV). HTLV infection converted the clone from a truly antigen-specific, major histocompatibility complex (MHC)-restricted inducer of B cell function to a line which indiscriminantly induced polyclonal Ig production in an MHC-nonrestricted manner. Clonal studies of T cells from patients with the acquired immunodeficiency syndrome (AIDS) were performed, and it was demonstrated that AIDS patients have markedly decreased cloning efficiency of both T4 (helper/inducer) and T8 (cytotoxic/suppressor) subsets. However, the subsets that were in fact clonable functioned normally. B cell lines from patients with inherited disorders of phagocytosis were developed and characterized making available unlimited numbers of cloned cells for biochemical and genetic study.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00212-04 LIR

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Study of the Immunopathogenic Features of Immune-Mediated Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: A. S. Fauci Chief LIR, NIAID

Others: J. B. Margolick Medical Staff Fellow LIR, NIAID
D. J. Volkman Senior Investigator LIR, NIAID

COOPERATING UNITS (If applicable) LIR/NIAID (J. I. Gallin); Clinical Pathology Department (H. Gralnick); Critical Care Medicine (J. E. Parrillo); Laboratory of Pathology (E. Jaffe); Mayo Clinic, Rochester, MN (G. Gleich); University of Florida, Gainesville (P. Katz); University of Washington, Seattle (W. Henderson).

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

4

PROFESSIONAL:

2

OTHER:

2

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We performed studies aimed at delineating the immunopathogenesis of a diversity of immune-mediated diseases and/or diseases characterized by aberrancies of immune function. We phenotypically characterized the T lymphocyte subsets infiltrating the thyroid glands of patients with Graves' disease and demonstrated that these glands were different from normals in that the follicular epithelium was infiltrated with a population of mononuclear cells comprised almost entirely of T cells with the T8 suppressor phenotype. We also studied the functional capabilities of T cells from Graves' disease patients at the clonal level. We determined the mechanisms for the hemophagocytosis observed in angiocentric lymphoproliferative diseases by demonstrating that malignant T cells from such patients secrete a phagocytosis inducing factor, which we have characterized and which activates human monocyte/macrophages to phagocytose. We developed Epstein-Barr virus (EBV)-transformed B cell lines from patients with the Chediak-Higashi syndrome and chronic granulomatous disease and demonstrated that these B cell lines manifested the precise metabolic defects as the phagocytic cells in these diseases. We identified and purified to homogeneity the components of the human eosinophilic granule including major basic protein, eosinophilic protein, and eosinophil-derived neurotoxin. These factors play a major role in the pathogenesis of the tissue damage in the idiopathic hyper eosinophilic syndrome. We delineated the potential mechanisms of the B cell hyperactivity in Sjogren's syndrome and initiated studies on the natural history, pathogenesis, and therapy of idiopathic dilated cardiomyopathy.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00213-04 LIR

PERIOD COVERED

October 1, 1983 to September 30, 1984

*TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical, Immunopathogenic, and Therapeutic Studies in the Spectrum of Vasculitis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: A. S. Fauci Chief LIR, NIAID

Others: D. J. Volkman Senior Investigator LIR, NIAID
H. C. Lane Senior Investigator LIR, NIAID

COOPERATING UNITS (if any)

Critical Care Medicine, NIH (J. E. Parrillo); Georgetown University (T. R. Cupps).

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

2

PROFESSIONAL

2

OTHER

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The LIR is currently studying prospectively the largest group of patients with the vasculitic syndromes in the world. On the basis of clinical, pathophysiologic, immunopathogenic and therapeutic results obtained over the past 16 years, we have designed a revised categorization scheme for the vasculitides which has now reached worldwide acceptance. We have developed and instituted aggressive chemotherapeutic regimens consisting of chronically administered cyclophosphamide together with alternate-day corticosteroids in several, formerly universally fatal diseases such as Wegener's granulomatosis. In this regard, we are now following over 100 patients with Wegener's granulomatosis in which we demonstrated a 95% remission and cure rate. We have now applied these approaches with remarkable success to other of the vasculitic syndromes such as systemic vasculitis of the polyarteritis nodosa group, isolated central nervous system vasculitis, Takayasu's arteritis, and the acute vasculitis of Sjogren's syndrome. In addition, we studied the pathophysiology of lymphomatoid granulomatosis and have shown it to be a prelymphomatous condition which responds in its early stages to chemotherapeutic regimens used in the vasculitic syndromes. Patients who responded in the early stage did not go on to lymphoma and remained disease free over long-term follow-up. The patient populations studied in the vasculitis protocol have been utilized to precisely delineate aberrancies of lymphocyte activation and immunoregulation seen in these diseases. In addition, the precise effects of various therapeutic regimens, particularly corticosteroids and cytotoxic agents, on human lymphoid cells have been described. In this regard, we demonstrated the exquisite and selective sensitivity of certain phases of the B cell cycle to cyclophosphamide therapy, an observation which might help explain its efficacy in certain diseases characterized by hyperreactivity of B cell function.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00390-01 LIR

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Studies of the Acquired Immunodeficiency Syndrome

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: H. C. Lane Senior Investigator LIR, NIAID

Others: A. S. Fauci Chief LIR, NIAID

J. B. Margolick Medical Staff Fellow LIR, NIAID

COOPERATING UNITS (if any)

Critical Care Medicine, Clinical Center (H. Masur); Medicine Branch, NIH (E. Gelmann).

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

3

PROFESSIONAL:

1

OTHER:

2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

An intensive effort was directed at studying the epidemiologic, etiologic, immunologic, and clinical aspects of the acquired immunodeficiency syndrome (AIDS). Over 150 patients with this illness have been enrolled in programs at the NIH. The LIR has performed the immunologic monitoring on all these patients. These large-scale studies established the fact that those patients with Kaposi's sarcoma alone are at the healthiest end of the immunologic spectrum. The AIDS patients were characterized as having marked quantitative and qualitative defects in cell mediated immunity. A selective defect in the ability of the helper T cell to respond to soluble antigen was delineated which provided an early clue to the T cell tropic nature of the subsequently uncovered etiologic agent. Examination of the B cells of these patients revealed a marked degree of polyclonal activation with a refractoriness to the normal signals for in vitro B cell activation coupled with an enhanced responsiveness to B cell growth factors. A series of clinical studies were carried out attempting in vivo to correct the immunodeficiency of these patients. Trials of both natural product gamma interferon and interleukin-2 were completed. While unable to effect an alteration in the clinical course of the patients, these trials revealed much concerning the pharmacokinetics of these new agents and their ability to modulate the human immune system. Peripheral lymphocyte transfers were carried out in 2 sets of identical twins, in each case one with AIDS and one healthy. In both instances only partial immune reconstitution was noted to occur, suggesting persistence of the AIDS agent. Finally, a variety of studies were initiated comparing various diagnostic techniques and therapies for several of the infectious complications of AIDS, particularly Pneumocystis carinii pneumonia and disseminated Mycobacterium avium-intracellulare.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00381-01 LIR

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Therapeutic Modalities in the Acquired Immune Deficiency Syndrome (AIDS)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: A. S. Fauci

Chief

LIR, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

PROFESSIONAL:

OTHER

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(a) Human subjects



(b) Human tissues



(c) Neither



(a1) Minors



(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Project terminated

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00382-01 LIR

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Delineation of the Immunological Defects in AIDS

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: A. S. Fauci Chief

LIR, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

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- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Project terminated



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LABORATORY OF INFECTIOUS DISEASES

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SUMMARY STATEMENT

Annual Report

Laboratory of Infectious Diseases
National Institute of Allergy and Infectious Diseases
October 1, 1983 to September 30, 1984

Historical Background

The LID is possibly the oldest Laboratory within the National Institutes of Health because it is the lineal descendant of the Public Health Service Laboratory of Hygiene founded in 1887. The Laboratory of Hygiene was directed to define the cause and epidemiology of medically important infectious diseases and to develop means for their control. This mandate has passed unmodified to LID through its germ line.

During the early 1960's the Respiratory Viruses Section of LID was concerned primarily with discovery of new respiratory pathogens and elucidation of their epidemiology. The human respiratory syncytial virus (RSV), which I had isolated the year before joining LID, was identified as the most important respiratory tract pathogen of early life and its natural history was charted in collaboration with Dr. Kapikian of LID and Dr. Robert Parrott of the Children's Hospital National Medical Center, Washington, D.C. The parainfluenza viruses were isolated for the first time during this interval and their importance in croup and other respiratory tract syndromes of childhood was established. Also, the agent of cold agglutinin pneumonia was shown to be a mycoplasma, Mycoplasma Pneumoniae. The natural history of this agent was elucidated and pneumonia caused by M. pneumoniae was shown to respond to tetracycline therapy.

By 1966, most of the major epidemiologic features of RSV, the parainfluenza viruses, adenoviruses, rhinoviruses coronaviruses and M. pneumoniae were established. In addition, host resistance to respiratory tract disease had been studied and this led to the demonstration that local mucosal immunity played a major role in resistance to parainfluenza virus, RSV and rhinovirus disease involving the upper respiratory tract.

New Direction - A Shift From Descriptive Epidemiology: Following collection and synthesis of considerable data from descriptive clinical and epidemiologic studies we gained a reasonably thorough understanding of acute respiratory tract disease. However, this new understanding was not matched by a decrease in incidence of disease. For this reason we made a commitment to redirect some of our future efforts to a more active mode and apply our newly acquired insights to the development of effective means for prevention of respiratory disease. As new programs in hepatitis virus and enteric virus disease research were initiated in LID this commitment became an integral part of their original mandate.

Initial Attempts at Immunoprophylaxis: Our first effort at prophylaxis was highly successful. In a series of volunteer studies Dr. Couch and I demonstrated that oral administration of adenovirus type 4 or type 7 enclosed in an enteric coated capsule induced a silent, immunizing infection

that remained localized to the lower intestinal tract. Virus did not spread to the upper respiratory tract which is the usual site of pathology. During field trials of the enteric vaccine in marine recruits we observed that it was essentially 100% effective in protecting against adenovirus disease during an extensive epidemic. Furthermore, virus did not spread from vaccinees to close susceptible contacts. Enteric adenovirus vaccine based on our original formulation is now licensed and is currently being used to prevent epidemic adenovirus disease in the military. Unfortunately, it was not possible to apply the technique of selective enteric infection to the control of other respiratory viral pathogens.

Use of Virus Genetics for Production of Attenuated Mutants: Following the realization that enteric immunization could not be used for other respiratory viruses, we initiated a program of virus genetics during the late 1960's with the intent of developing attenuated mutants for use in immunoprophylaxis. Initially, conditional lethal, temperature sensitive (ts) mutants of RS and influenza A viruses were produced, characterized genetically and then evaluated for evidence of attenuation in experimental animals and subsequently in volunteers. A satisfactorily attenuated and antigenic ts mutant of RS virus was identified during collaborative studies with Dr. Parrott of the Children's Hospital National Medical Center, Washington, D.C. Satisfactorily attenuated and antigenic ts mutants of influenza A virus were also produced by Dr. Murphy (LID). Unfortunately, instability of the attenuation phenotype during infection of susceptible individuals proved to be an insurmountable problem. Although illness did not develop in seronegative volunteers, on occasion these individuals shed virus that had lost its ts property. As shown by Dr. Murphy, loss of the ts phenotype resulted from second site ("suppressor") mutation. This experience led us to adopt an alternate strategy for attenuation of respiratory viruses that will be discussed later.

New Programs in Hepatitis and Gastroenteritis: Eighteen years ago an LID program in hepatitis viruses was initiated because it was believed that the newer techniques that had proved successful in uncovering fastidious respiratory viruses might permit detection of hepatitis viruses that had eluded research virologists for at least 20 years. This did not prove to be the case. Instead, other approaches were required to unmask these viruses. These approaches also proved successful in detecting the two major groups of enteric viral pathogens that were prime targets of LID's gastroenteritis program that began in 1970. Collectively, these approaches can be described as "particle virology", i.e., the study of viruses as non-replicating particles that possess specific antigens and exhibit a distinctive morphology.

Four groups of viruses were initially detected as non-replicating virus-like particles that could not be cultivated in vitro. The first virus was hepatitis B virus (HBV) that was detected by immunodiffusion and direct electron microscopy by Dr. Blumberg (Philadelphia) and Dr. Prince (New York Blood Bank). HBV was first detected in the form of HBV surface antigen (HBsAg) that was present in high concentration in the plasma of infected individuals. Subsequently, the virion of HBV was identified by electron microscopy by Dr. Dane (UK) as an unusual morphologic virus particle present in the plasma of HBV infected individuals in a concentration considerably lower than HBsAg.

The next virus to be detected by the methods of "particle virology" was the Norwalk virus, a major cause of epidemic acute non-bacterial gastroenteritis. Initially, in volunteer studies performed in collaboration with Drs. Blacklow and Dolin (LID) the etiologic agent of the Norwalk outbreak was detected in a bacteria-free stool filtrate derived from a patient who became ill during the outbreak. This filtrate induced the disease which was then transmitted serially using stool filtrate from ill volunteers. Subsequently, Dr. Kapikian (LID) identified the etiologic agent by immune electron microscopy (IEM) and demonstrated that it was a 27nm virus-like particle. This was the first time IEM had been used to detect a new viral pathogen.

Shortly thereafter, Dr. Kapikian together with Drs. Feinstone and Purcell of LID used IEM to visualize the virus of hepatitis A (HAV) and establish its relationship to epidemic hepatitis. This led to the recognition of non A, non B hepatitis by Drs. Purcell, Feinstone and their colleagues in LID and the NIH Blood Bank. Non A, non B hepatitis virus(es) presently cause most post transfusion hepatitis.

Finally, rotavirus, the major virus of severe infantile diarrhea, was initially visualized in duodenal biopsies by Dr. Bishop in Australia. Very shortly thereafter, it was detected in abundance in the stools of infants with diarrhea. This was accomplished by direct electron microscopy almost simultaneously by investigators in Australia, the UK, Canada and the U.S. Rotavirus was first detected in the U.S. by Dr. Kapikian.

Surprisingly, only two of the viruses just described, HAV and rotavirus, were ultimately cultivated *in vitro*. All attempts to grow HBV and Norwalk virus in tissue culture have failed. Nonetheless, the natural history and molecular biology of HBV is now well understood and effective first and second generation subunit (HBsAg) vaccines have been developed. Dr. Purcell and Dr. Gerin (Georgetown University) developed one of the earliest purified HBsAg vaccines with antigen derived from the plasma of chronic carriers. They were the first to demonstrate protective efficacy of such a vaccine in chimpanzees.

Similarly, the basic epidemiology of the Norwalk group of enteric viruses is now understood from studies that employed the techniques of "particle virology". Most of this knowledge has come from studies by scientists in LID (Drs. Kapikian and Greenberg) who developed second and third generation serologic tests and applied them to an investigation of epidemics of acute gastroenteritis.

Shift Toward Molecular Biology: More recently a major commitment was made to add a strong molecular biologic presence to LID that would complement and strengthen ongoing programs in epidemiology, virus genetics and experimental immunophylaxis. Drs. Lai, Markoff and their colleagues began their molecular biologic manipulation of influenza virus genes 5 years ago. cDNA clones of 6 genes (PB2, HA, NA, MP, M and NS) were prepared and shown to be complete by sequence analysis. Subsequently, 5 of the complete cDNA clones were expressed in eukaryotic cells using an SV40 vector into which cloned influenza DNA was inserted into the late region. Functional influenza proteins were produced in these cells and this set the scene for analysis of functional domains of viral proteins by deletion or by site specific

mutagenesis of defined regions of influenza genes. Using this approach, Drs. Lai and Markoff have delineated functional domains of the HA and NA surface glycoproteins.

During the course of a study of the two smallest influenza virus genes, Drs. Lai and Lamb (Rockefeller University) detected the first evidence for splicing of mRNA transcribed from an RNA gene. This was observed for the M and NS genes that each produce 2 or 3 separate RNA transcripts, one being colinear with the gene and the other(s) representing a spliced product.

Recently molecular biologic techniques have also been applied to a study of the rotavirus genome by Dr. Flores and his colleagues in LID. He observed that the vast majority of human rotaviruses fell into two families as indicated by cross RNA hybridization. Furthermore, the 11 genes of human rotaviruses belonging to these two families were found to be quite distinct from the corresponding genes of rotaviruses recovered from various animal species. Full length or nearly full length clones of the 9 largest genes of three rotaviruses (a human, a simian and a bovine strain) have been constructed. The gene that codes for the major neutralization protein of the human rotaviruses is almost completely sequenced and the corresponding genes of the animal rotaviruses are not far behind.

Following the realization that the attenuation phenotype of RS virus ts mutants bearing a limited number of point mutations could be easily suppressed by second site mutations, the decision was made to abandon this approach to vaccine development. At this point, we decided to redirect our efforts in the RS virus program toward gaining a better understanding of the viral genome at the molecular level. Dr. Venkatesan and his colleagues have made considerable progress in this direction during the past 2 1/2 years. Each of the RS viral genes has been cloned and approximately 70% of the entire 15 kb RNA genome has been sequenced. Unusual features of gene organization that differentiate RS virus from other nonsegmented negative strand RNA viruses (rhabdoviruses and paramyxoviruses) have been identified by sequence analysis.

Finally, the latest program in molecular biology involves HAV which was cloned successfully by Drs. Ticehurst, Baroudi, Feinstone and Purcell. A nearly complete genetic map has been constructed from overlapping clones that represent the entire genome except for a region of approximately 30 bases at the 5' end. More than half of the genome has been sequenced and a single long open reading frame was identified.

ACUTE VIRAL GASTROENTERITIS

Rotavirus

Rotavirus Genetics: Rotaviruses, newly classified members of the Reoviridae family, are an important cause of infantile diarrhea in a wide variety of mammalian species including man. In fact, rotaviruses have emerged as the single most important cause of severe infantile diarrhea throughout the world.

Because they have a segmented genome, rotaviruses undergo genetic reassortment at high frequency during mixed infection. Previously rotavirus

reassortants were used to establish gene coding assignments for several major antigenic specificities and functional activities of these viruses. During the last year we succeeded in isolating a large number of reassortant viruses derived from coinfection of a fastidious "non-cultivable" human rotavirus (strain D [serotype 1], strain DS-1 [serotype 2], strain P [serotype 3], or strain ST-3 [serotype 4]) and a wild type bovine or a rhesus rotavirus. These reassortants were isolated specifically as vaccine candidate strains. Wild type animal rotaviruses were used since these viruses are less likely to have silent point mutations than mutagenized ts virus that had served as a parent in previous reassortment experiments. Silent single point mutations are generally unstable and tend to confound genetic analysis of attenuation. Two selection procedures were used for isolation of the desired reassortants. First, fastidious (i.e., "non-cultivable") human rotavirus strains were employed as the source of human rotavirus genes. Second, antiserum or a monoclonal antibody mixture specific for the animal rotavirus 38Kd VP₇ glycoprotein was employed to neutralize viruses that exhibited the serotype of the animal rotavirus parent. When this strategy was employed with either RRV or bovine rotavirus as the animal rotavirus parent, we succeeded in isolating reassortants with the neutralization specificity of the type 1, type 2, type 3, or type 4 human rotavirus. Analysis of the genotype of these reassortants indicated that the majority of the reassortants possessed 10 genes of animal rotavirus origin and a single gene of human rotavirus origin, namely the gene that codes for the major neutralization protein (VP₇). These single human rotavirus gene substitution reassortants exhibit the serotype specificity of their human rotavirus parent as determined by plaque reduction neutralization assay.

Reassortants with a single human rotavirus gene substitution represent promising candidate live vaccine strains. The narrow and highly restricted host range observed for rotaviruses recovered from a wide variety of species predicts that substitution of a large number of animal rotavirus genes for the corresponding genes of human rotavirus should lead to attenuation of reassortants for humans. On the other hand the major protective antigen is derived from the human rotavirus parent and hence the reassortant will exhibit the serotype of a human rotavirus. Recently single human rotavirus gene substitution reassortants were adapted to growth in DBS FRhL-2 cells. Successful passage in this diploid cell strain further enhances their potential as vaccine candidate strains (Midthun, Kapikian, Chanock).

Development of rotavirus vaccines: Within the past year a variety of human and animal rotaviruses have been grown in cell culture and are available for further study as potential vaccine candidates. In a previous study in adult volunteers immunologic correlates of resistance were defined. In the current studies these correlates (serum antibodies measured by a variety of techniques, the most important being neutralization in tissue culture) were used to select volunteers for evaluation of candidate vaccine strains. Our intent was to identify rotaviruses that infected susceptible adults, induced immunity, but did not cause disease. Three candidate viruses were evaluated, the human Wa rotavirus (serotype 1) and two animal rotaviruses, the bovine UK rotavirus and the rhesus rotavirus (RRV). The human Wa rotavirus and the rhesus rotavirus infected susceptible adult volunteers and induced a rotavirus antibody response but these viruses did not cause disease. The bovine UK rotavirus did not appear to infect adult volunteers

suggesting that perhaps it was too restricted to serve as a vaccine virus in man.

The rhesus rotavirus (RRV) appears to be particularly promising because it induced an appreciable homologous antibody response in 29 of 31 young adults who had a very low level of serum neutralizing antibody. However, it did not cause any recognizable signs or symptoms of disease. It should also be noted that the rhesus rotavirus is similar to the serotype 3 human rotavirus by neutralization. Furthermore, rhesus rotavirus stimulated heterotypic serum antibody responses to type 1, type 2, and type 4 human rotaviruses in a significant proportion of the adult volunteers. Finally, rhesus rotavirus may prove useful as a donor of attenuating genes to reassortant viruses that bear the major neutralization protein (VP₇) of a human rotavirus belonging to serotype 1, 2, or 4. In this manner homotypic protection could be provided for each of the human rotavirus serotypes. Indeed, single gene substitution reassortants are now available for each of the human rotavirus serotypes. These viruses possess 10 rhesus rotavirus genes and a single human rotavirus gene, the one that codes for neutralization specificity. It is likely that such single gene substitution reassortants will induce immunity to viruses belonging to the serotype of their human rotavirus parent while at the same time they should retain the attenuation of their rhesus rotavirus parent.

We are also encouraged by these studies with RRV since we now have convincing evidence that the transaminase elevations observed with this strain (and other attenuated strains) in volunteers represent random events not related to the rotavirus inoculum. Data from other studies at the Center for Vaccine Development support this conclusion (Kapikian, Wyatt, Midthun, Glass, Chanock).

Molecular Biology and Epidemiology of Rotaviruses.: cDNA copies of genes of three different rotavirus strains (the human Wa [serotype 1], the bovine NCDV and the simian rhesus Rh2 [serotype 3]) were prepared by reverse transcription of their genomic RNAs or of single stranded RNAs synthesized in vitro from rotavirus particles. The cDNAs were tailed and inserted into pBR322 and the resulting recombinants were used to transform E. coli. The genes from which most of these clones were derived were identified by means of a dot hybridization assay. Clones representing each rotavirus gene (except for genes 10 and 11) have been identified. The size of the rotavirus cDNA inserts in the recombinant plasmids were determined for more than half of the 2500 clones obtained.

Clones representing genes coding for the major neutralization antigen (the 34-38,000 dalton VP₇ glycoprotein) of the three strains were identified. Inserts that appeared to be full size were mapped by restriction endonuclease analysis. Interestingly, 21 of the clones representing the Wa gene had a similar size, the same restriction map and the same orientation in the plasmid. The presence of two unevenly spaced Hinf I sites on the gene 9 cDNA has allowed 5' labeling of three fragments of this gene for initial sequencing. Preliminary comparisons have been made with sequences recently published for the corresponding VP₇ genes of simian rotavirus strain SA-11 and bovine rotavirus strain UK, which represent serotypes different from each other and from the human rotavirus Wa. The strong degree of homology between sequences of the SA-11 and UK VP₇ genes is

also shared by the regions already sequenced in the Wa virus (about 90% of the gene). Long stretches of amino acid homology were shared by the three viruses and this was interrupted occasionally by isolated divergence of a single amino acid or in a few instances by clusters of amino acid changes. One such cluster of amino acid substitutions is located near the amino terminal end; however, this area is strongly hydrophobic, and hence it probably does not represent an antigenic determinant. The strong hydrophilic area near the carboxyl end of the molecule, postulated as a strong antigenic determinant for the SA11 neutralization protein, may not be responsible for serotypic differences because the amino acid sequences in that area are shared by the three strains. Other highly hydrophilic regions in VP₇ do not exhibit major differences among the three genes. It is possible that only a few amino acid substitutions may produce major conformational changes in the molecule which in turn are responsible for serotypic differences. Recognition of such sites may be possible when we complete our sequence analysis, and examine the sequences of VP₇ genes from viruses belonging to other serotypes; we are currently sequencing the VP₇ genes of NCDV and rhesus rotavirus Rh2. Alternatively, the expression of VP₇ (or portions of it) in a eukaryotic cell system followed by analysis with monoclonal antibodies that exhibit neutralizing activity may facilitate identification of major antigenic sites (Flores, Glass, Nakagomi).

Rotavirus single stranded (ss) RNA probes prepared by in vitro transcription of purified virions were used in a dot hybridization assay to detect the presence of rotavirus in stools and other biological materials. The assay is highly specific and sensitive and its use may facilitate epidemiological studies of rotavirus gastroenteritis. The assay is able to detect as little as 8 pg of purified double stranded RNA in a homologous reaction. The dot hybridization assay was 10-100 times more sensitive than ELISA for detection of rotavirus. The specificity of the assay has been confirmed repeatedly by the lack of reactivity of the probes with known negative samples. Recently, we have employed probes obtained from recombinant plasmids carrying rotavirus cDNA copies derived by reverse transcription of rotavirus RNAs. These probes are easier to produce and to label, but their sensitivity for detection of rotavirus is less than that of the ss RNA probes.

ss RNA probes have also been employed successfully for analyzing and quantitating the degree of genetic relatedness among different rotavirus strains of human and animal origin. For example, cloned DNA representing the Wa rotavirus VP₇ gene, which encodes the major neutralization antigen, was useful in studying genetic relatedness among different strains. When heat-denatured stool specimens or tissue culture samples were dotted on nitrocellulose membranes and hybridized to a Wa gene 9 probe, it was possible to distinguish rotaviruses belonging to other serotypes (including strains of serotype 2 and serotype 4, and many but not all strains of serotype 3) from the Wa strain and other type 1 rotaviruses as well. This approach may provide a method to serotype rotaviruses without the need for cultivation.

Cloned DNA representing the Wa rotavirus gene 4 has also been useful in studying the molecular epidemiology of rotaviruses. Nick translation probes were used in a dot hybridization assay to study homology of the Wa strain to other human rotaviruses as well as a series of animal rotaviruses.

Preliminary results indicated that the gene 4 probe recognized similar sequences in most of the human rotavirus strains tested but the probe did not react significantly with animal rotavirus strains. This suggests that the product of this gene may be involved in host range restriction. Evidence for a role of the fourth gene product in host range was obtained previously during a study of "non-cultivable" human rotavirus X cultivatable animal rotavirus reassortants. In each instance cultivatable reassortants acquired their fourth gene from the cultivatable bovine virus. None of the other genes showed this strict association with cultivability in vitro. Differences in the sequence of this gene may be responsible for the known host specificity of different viral strains (Flores, Glass, Nakagomi).

Cultivation of rotaviruses: The need to define antigenic differences among human rotaviruses led us to cultivate directly in cell culture a variety of rotavirus strains from diverse geographical areas and populations. Over 90 strains of human rotavirus have been cultivated in MA104 or African green monkey kidney (AGMK) cells. Four distinct serotypes of human rotavirus were identified and compared with each other by plaque reduction or tube neutralization assay. Based upon these findings a numbering system which includes both human and animal rotaviruses was proposed (Hoshino, Wyatt, Kapikian).

Norwalk-like Viruses of Acute Epidemic Non-bacterial Gastroenteritis: The Marin County agent is a 27 nm virus-like particle which was associated with two separate outbreaks of nonbacterial gastroenteritis in northern California in 1978 by L. Oshiro. The agent is morphologically similar to but serologically distinct from the Norwalk, Hawaii and Snow Mountain agents when studied by immune electron microscopy (IEM) and solid phase radioimmunoassay (RIA) antibody blocking assay. One ml of a safety tested, bacteria-free filtrate prepared from a stool specimen from one of the individuals ill during the original Marin County outbreak was administered orally to seventeen adult volunteers. None of these individuals developed definite clinical illness. Two additional volunteers were later fed 20 ml of the filtrate. One of these volunteers developed a gastrointestinal illness characterized by nausea, vomiting, diarrhea and malaise. Interestingly, this illness started five days after administration of the fecal filtrate and lasted 36-48 hours. Many 27 nm particles were visualized in diarrheal stools from the ill volunteer. These particles were shown to be identical to the Marin County agent by IEM using acute and convalescent sera from the original outbreak. A RIA was developed for the Marin County agent using as antigen the particle-rich stool of the ill volunteer. A preliminary survey of a series of gastroenteritis outbreaks by RIA failed to implicate the Marin County agent as an important cause of epidemic gastroenteritis (Midthun, Askaa, Kapikian).

Influenza A Virus

Attenuation of human influenza A virus by substitution of avian influenza "internal" genes: The major goal of this project is the construction and characterization of attenuated influenza A reassortant viruses for use in a live virus vaccine for man. In addition, the potential usefulness of other new approaches to immunoprophylaxis against influenza virus are being explored.

We had previously demonstrated that the avian influenza A/Mallard/NY/6750/78 virus could serve as a donor of attenuating genes to three different wild type human influenza A viruses. The three avian-human influenza A reassortant viruses each contained the hemagglutinin (HA) and neuraminidase (NA) genes of a human wild type influenza A virus while the six other ("internal") RNA segments were derived from the avian influenza A virus parent. The reassortant viruses replicated efficiently in tissue culture at 42°C, like their avian influenza virus parent, and were restricted in replication in the respiratory tract of monkeys and hamsters. This year we produced another reassortant using the A/Korea/1/82 strain as the wild type human influenza A virus parent, and the A/Mallard/78 strain as the avian influenza A virus parent. This reassortant has the genotype and biological properties of the three other reassortants tested previously. This new reassortant is currently being evaluated for safety and immunogenicity in humans (Murphy, Snyder, Buckler-White, Chanock).

The suitability of other avian influenza A viruses as donors of attenuating genes is currently being evaluated. One virus, the A/Pintail/119/79 (H4N6) avian strain has been mated with the human A/Washington/80 (H3N2) wild type virus and a reassortant virus with the A/Washington/80 surface proteins and the six "internal" genes of the avian virus has been isolated. This reassortant virus replicates efficiently at 42°C; in this property it resembles its avian influenza A virus parent. The reassortant is also restricted in its growth in monkeys; in this respect it is also similar to its avian influenza A virus parent. This reassortant is currently being evaluated in humans for safety and immunogenicity.

We have chosen two other avian viruses for evaluation as donors of attenuating genes. One virus, A/Mallard/Alberta/573/78 (H1N1), failed to replicate in the lower respiratory tract of squirrel monkeys. The other strain, A/Mallard/Alberta/88/76 (H3N8), replicates only 10-40 times less well than human influenza virus in the squirrel monkey's lower respiratory tract. We are producing a series of four avian-human influenza reassortant viruses containing the HA and NA of the human influenza A/Korea/1/82 virus while the "internal" genes are derived from one of the four avian influenza A viruses described above. These four avian-human influenza A reassortants will be studied systematically in squirrel monkeys, in adult volunteers, and (if safe in adults) in seronegative children to determine which avian influenza A donor virus confers on avian-human influenza A reassortant viruses the optimal balance between attenuation and immunogenicity. The genes responsible for attenuation in each of these avian viruses will also be evaluated.

An avian influenza A virus, A/Mallard/NY/6750/78 (H2N2), was previously shown to be restricted in replication in the respiratory tract of squirrel monkeys. Avian-human influenza A reassortant viruses possessing the six RNA segments coding for non-surface proteins (i.e., "internal" genes) of this avian virus were as restricted in replication in squirrel monkeys as their avian influenza A virus parent. These findings indicate that restriction of replication of the avian influenza donor virus is a function of one or more of its "internal" genes. To investigate which of the avian influenza A virus genes or combination of genes was responsible for diminished replication in the respiratory tract of primates, we produced reassortant viruses that contained the human influenza virus surface antigens of the

A/Udorn/72 (H3N2) virus while one or more of the internal genes were derived from the avian influenza virus parent. Avian-human influenza A reassortant viruses that derived only the nucleoprotein (NP) or matrix (M) protein gene from the avian influenza parent were as restricted in their growth as an avian-human influenza reassortant virus that possessed all six avian influenza "internal" genes. In addition, an avian-human influenza reassortant virus possessing only the avian RNA 1 and NS genes (which by themselves do not specify restricted replication) exhibited significant restriction of virus replication in the trachea of the squirrel monkey. Thus, the avian influenza NP and M genes appear to play a major role in host range restriction of the avian influenza A/Mallard/78 virus and its reassortants, but the combination of avian influenza RNA 1 and NS genes also contributes to restriction of replication.

The findings from the present study have important implications for the use of avian influenza A viruses as donors of genes for attenuation of human influenza A viruses. Because the avian influenza NP and M genes are each able to attenuate a human influenza A virus for monkeys, restoration of virulence of an avian-human influenza virus reassortant will require appropriate genetic changes in both genes or suppression of both genes. Previously it was observed that an influenza A virus with ts mutations affecting two separate genes was able to escape its attenuation phenotype after replication in experimental animals or susceptible volunteers. It is likely that the divergence of amino acid sequence of the the avian influenza M and NP proteins from that of their human influenza virus counterparts is considerably more extensive than that of influenza virus ts mutants from their wild type virus parent. If this prediction is correct, it is unlikely that avian influenza genes could easily develop the number of mutations required for restoration of virulence, especially during a restricted infection of susceptible individuals. Also the avian influenza RNA 1 and NS genes contribute to attenuation and this provides an additional degree of stability to avian-human influenza reassortant viruses bearing the six "internal" avian influenza genes. Studies are being carried out in man and in monkeys to assess the level of genetic stability conferred on avian-human influenza reassortant viruses by the six avian A/Mallard/NY/6750/78 "internal" genes (Murphy, Snyder, Buckler-White, Chanock).

The sequence of the A/Mallard/78 M gene has been determined and compared to that of the M gene of a human influenza A virus. Relatedness of the two genes at the amino acid level was 96% for the M1 cistron but only 86% for the M2 cistron. This indicates that a significant sequence divergence exists in the M2 cistron. This is probably also the case for the NP gene because synthetic oligonucleotide primers that are currently being used to sequence the human influenza NP gene do not prime synthesis of cDNA from the avian influenza A/Mallard/78 NP gene. Such extensive sequence divergence should increase the stability of the attenuation phenotype over that observed previously for the ts mutant reassortants (Buckler-White, Murphy).

Clinical evaluation of avian-human influenza A virus reassortants: An influenza A reassortant virus that derived its genes for the surface glycoproteins from the human influenza A/Washington/897/80 (H3N2) virus and its six other RNA segments from the avian influenza A/Mallard/6750/78 (H2N2) virus was evaluated for safety and immunogenicity in 82 seronegative adult

volunteers. Only two of 51 (4%) infected vaccinees developed an illness (brief systemic symptoms without fever), whereas 11 of 24 (46%) volunteers who received wild-type human influenza A/Washington/897/80 virus became ill. The live virus vaccinees shed significantly less virus than did volunteers infected with wild type human influenza virus, a finding consistent with the greatly reduced reagentogenicity of the vaccine virus. Replication of the avian-human influenza reassortant virus appeared to be confined to the respiratory tract because virus was not recovered from the blood or stools of the vaccinees. The 50% human infectious dose (HID_{50}) of the reassortant was $10^{5.9}$ TCID₅₀. At 50 to 100 HID_{50} 's 80 to 89 percent of the vaccinees developed an antibody response. These findings indicate that the avian-human A/Washington/80 influenza reassortant virus is safe and immunogenic in adults. These observations also provide the basis for further evaluation of this and other similarly derived reassortants in humans (Murphy, Snyder).

Clinical evaluation of cold adapted (ca) human influenza reassortant viruses: Seronegative adult volunteers who received live cold-adapted influenza [A/Washington/80 (H3N2) or A/California/78 (H1N1)] vaccine with 6 "internal" ca genes or a trivalent inactivated vaccine were challenged 6 months later with homologous wild type human influenza virus. The live and inactivated virus vaccines provided protection against systemic and febrile illness: 91% efficacy for the live virus vaccine (pooled data for H1N1 and H3N2 vaccinees) and 75% efficacy (also pooled data) for inactivated virus vaccine (Murphy, Snyder).

The cold-adapted (ca) A/Ann Arbor/6/60 donor virus has been shown to reproducibly confer attenuation on wild type human influenza A viruses through transfer of its six "internal genes" (genes coding for proteins other than the hemagglutinin and neuraminidase). However, the specific ca A/Ann Arbor/6/60 internal gene(s) responsible for the attenuation phenotype have not been defined. Previous studies in man have shown that the ca genes coding for the nonstructural (NS), matrix (M), and PB1 polymerase proteins are each not essential for attenuation. Each ca reassortant virus previously shown to be attenuated in man contained the ca PB2 and PA polymerase genes and the nucleoprotein (NP) gene, suggesting that one or more of these genes is necessary for attenuation. We have evaluated a new reassortant which contains the PB1 polymerase and M genes from the ca parent and all other genes from an influenza A/Washington/879/80 (H3N2) wild type virus. This reassortant displays the ca phenotype and is intermediate between its two parents in its level of temperature sensitivity in vitro and in attenuation for ferrets. The virus failed to produce illness in 14 seronegative adult volunteers inoculated intranasally with $10^{7.0}$ TCID₅₀. Virus shedding was detected in 2 of 14 volunteers. This level of attenuation is similar to that exhibited by reassortants containing all six ca internal genes. Although the PB1 and M genes were individually not essential for attenuation in previous studies, a combination of these genes has now been shown to produce attenuation. In the context of previous observations, the current results suggest that the attenuation of ca reassortant viruses is a relatively complex phenomenon in which probably at least 3 ca genes play a role (Snyder, Murphy).

Molecular Biology of Influenza A virus

Strategy of transcription of the influenza A virus NS gene: The genome of influenza A virus consists of eight single-stranded RNA segments that are transcribed in infected cells by the virion transcriptase into their corresponding mRNA's. Influenza virus RNA segment 8 codes for two distinct proteins, NS₁ and NS₂, that are translated from separate mRNA's. Mapping and sequence studies have shown that the NS₁ mRNA is a colinear transcript and NS₂ mRNA contains a spliced region. In order to investigate the splicing potential of influenza virus mRNA derived from virion RNA segment 8, cloned full-length NS DNA was inserted into the late region of an SV40 expression vector and the recombinant was used for infection of primate cells. S1 nuclease mapping of transcripts and determination of their nucleotide sequence indicated that both interrupted and uninterrupted mRNA's containing influenza NS sequences were synthesized in cells infected with the recombinant. The sequences found at the junction of the interrupted mRNA were identical to those found in the NS₂ mRNA produced in influenza virus infected cells. These observations indicate that processing of the NS mRNA transcript during influenza virus infection involves a mechanism of splicing similar to that which occurs with DNA-directed RNA transcription. Our observations thus eliminate other possible explanations for interrupted mRNAs such as transcription from defective interfering particles and "transcriptional jumping" (Lai).

Expression of influenza A virus neuraminidase NA glycoprotein from cloned DNA: The functional NA molecule is a tetrameric structure projecting outward from the surface of influenza virus infected cells and from virus particles. The four identical glycosylated monomeric components are inserted across the membrane and are anchored by their N-terminus. Sequence data from our laboratory and from several other laboratories indicates that the NA monomer does not have a C-terminal hydrophobic region of sufficient length (20-25 amino acids) to span a membrane. In this respect, the NA differs from most other well-studied eukaryotic glycoproteins, such as the influenza HA, the VSV G protein, membrane-bound immunoglobulins, and the major histocompatibility antigens. Also, cleavage does not occur in the N-terminal region during processing of the nascent NA polypeptide in contrast to the foregoing membrane glycoproteins which have an N-terminal hydrophobic "signal sequence" that is cleaved.

A full-length ds DNA copy of the virion RNA segment coding for an influenza A neuraminidase (NA) glycoprotein was previously cloned into the late (deleted) region of an SV40 shuttle vector. The influenza-specific product of a lytic infection with this vector was shown to be glycosylated and inserted in the outer cell membrane. Additional studies established that weak enzymatic activity of the vector-coded NA was detectable in lysates of infected cells. Three deletion mutant NA DNAs that lacked sequences coding for 7 (dIK), 21 (dII) or all 23 amino acids (dIZ) of the N-terminal hydrophobic region of the wild-type NA were constructed and characterized. A comparison of the phenotypes of these mutants suggested that this region functions not only in membrane anchorage but also as a signal sequence, permitting entry of the nascent NA polypeptide into membrane organelles for glycosylation. Experiments are now in progress to induce point mutations in DNA coding for the hydrophobic N-terminus of the NA protein to determine: (1) whether the membrane anchorage function and

the "signal sequence" function of this sequence can be altered separately and (2) whether the strict conservation of the N-terminal 12 residues in this region among all influenza A strains has special significance (Markoff, Lai).

Functional analysis of the influenza A hemagglutinin by site specific mutagenesis: The influenza virus hemagglutinin (HA) is an integral membrane glycoprotein that accumulates at the surface of infected cells and is projected as a spike from the virion surface. The HA plays a key role during viral infection, being responsible for initial attachment to the cell surface and subsequent fusion of the viral envelope with intracellular membranes. The nascent HA polypeptide contains two hydrophobic tracts of amino acid sequences: the carboxy-terminal hydrophobic region that is responsible for anchoring the protein in the cell membrane and the amino-terminal hydrophobic region that serves as a signal in the process of glycosylation, transport, and surface expression. In eukaryotic cell systems, specific interactions of the signal peptide with endoplasmic reticulum membranes are necessary to initiate translocation of the nascent polypeptide across the intracellular membranes.

We employed site-specific mutagenesis to generate a series of mutants of an influenza HA-SV40 recombinant. These mutants contained point mutations in the region of the HA gene that encodes the signal peptide sequences. The mutant HA-SV40 recombinants were then used to transfect African green monkey kidney cells in order to achieve expression of mutant hemagglutinins. Characterization of mutant influenza virus HAs with altered signal peptide revealed that a majority of the mutations had no effect on functional properties of HA such as cell surface expression and erythrocyte binding. However, one mutant (designated mutant 28) that sustained multiple amino-acid substitutions produced HA that remained intracellular and was not expressed on the cell surface. A comparison of the sequence of this mutant with those of other mutants that are expressed normally on the cell surface suggested that amino acid substitution at the signal cleavage site was responsible for the observed functional abnormality. The defective intracellular HA contained an endoglycosidase H (endo H) sensitive carbohydrate component, whereas the endo H resistant sugar moiety normally present on the wild type HA was not detected. In addition, the molecular size of the non-glycosylated form of mutant 28 HA appeared to be larger than non-glycosylated wild type HA as indicated by a slower migration rate on SDS-polyacrylamide gel. This finding is consistent with the sequence data indicating that the mutant HA was altered at the signal cleavage site. These results suggest that mutant HA containing uncleaved hydrophobic signal sequences translocates across the microsomal membranes but fails to undergo further transport to the Golgi apparatus where additional processing of carbohydrate components takes place (Lai).

Attempts to rescue cloned influenza DNA by coinfection of transfected cells with influenza A virus: Deletion mutations offer an attractive alternative to other approaches to stabilization of attenuation. Such mutations should be stable because they are not subject to reversion and it is unlikely that they would be easily suppressed by a new mutation at another site on the viral genome. For these reasons we have initiated efforts to isolate or construct stable deletion mutations that will render influenza A virus sufficiently defective that it becomes attenuated but not so defective that

it loses viability. Viable deletion mutants of DNA viruses such as polyoma and HSV-1 have been constructed by others and some of these mutants have been shown to be attenuated in experimental animals.

Defined regions of an influenza A virus gene can be deleted using recombinant DNA techniques. This type of genetic surgery can only be performed on DNA. Hence, the RNA genes of influenza virus must be transcribed into complementary DNA and manipulated in this form. This presents us with the difficult problem of transcribing mutant DNA into an RNA form that can be transferred back into an infectious virus. This transfer has not been a problem with poliovirus which contains positive strand genomic RNA that is infectious. Cloned, full-length poliovirus cDNA was recently shown to be infectious in tissue culture. Unfortunately, influenza virus has a negative-strand RNA genome that is not infectious.

Efforts to produce stable deletion mutations in the genes of an H3N2 influenza A virus began with cloning full-length DNA copies of 6 of the 8 virus genes--PB2, HA, NA, NP, M and NS. Each of these cloned genes retained the conserved 5' and 3' terminal sequences. In addition, the NA, M and NS clones were sequenced and shown to contain the complete nucleotide sequence of their respective genes. Thus, these clones contain all the sequences required to derive corresponding RNA transcripts that possess the control signals for replication of viral genes.

We used SV40 as a vector to facilitate introduction and subsequent transcription of influenza viral DNA in mammalian cells. To test the possibility that the cloned DNA could be converted to vRNA and packaged in virions, we performed influenza gene rescue experiments, so called allele replacement, using recombinants of HA-SV40 and NA-SV40. These recombinants were used initially because of the ease of detection of the HA (H3) and NA (N2) surface antigens coded by cloned DNA and the availability of antisera which could neutralize virus bearing the HA (H1) or NA (N1) of the coinfecting influenza A strain. Permissive AGMK cells were co-infected with a HA-SV40 or NA-SV40 recombinant that produced messenger sense RNA transcripts and influenza A/WSN/33 (H1N1) virus which bore surface antigens of another subtype. In this situation gene rescue should be recognized easily by the presence in the growth yield of virus bearing either an H3 or N2 surface glycoprotein. We were unsuccessful in detecting rescue of the HA or NA gene. Evidence of gene rescue was also sought during co-infection with a recombinant of NP-SV40 and an influenza virus ts mutant which has its mutation in the NP gene. Rescue was not detected in experiments in which the NP-SV40 or NA-SV40 recombinant produced negative strand NP or NA RNA transcripts. Also rescue was not detected in experiments in which the NP-SV40 recombinant produced positive strand NP RNA transcripts.

Our failure to detect rescue suggested that the influenza transcripts were not replicated or encapsidated or packaged in virions. Sequence analysis indicated that influenza (+) RNA transcripts were flanked at their 5' and 3' ends by SV40 initiation and polyadenylation sequences respectively. Possibly these flanking SV40 sequences prevented recognition of influenza terminal sequences by the polymerase complex of the coinfecting influenza virus. Perhaps these specific terminal influenza sequences are also required for encapsidation of influenza virus RNA transcripts but are obscured by the flanking SV40 sequences (Markoff, Lai, Chanock).

Persistent expression of cloned influenza DNA and detection of spontaneous viable deletion mutations: Perhaps it may not be necessary to construct viable deletion mutations using cloned viral DNA. Recently, Dr. Maassab (U. of Michigan) and Dr. Palese (Mt. Sinai) identified a spontaneous 36 base, viable deletion mutation in the middle of the NS1 coding region of the 8th gene of an influenza A virus. This spontaneous deletion is of some interest because it produces a host range effect. Growth is restricted in mammalian cells but not in eggs or avian cells in culture. Currently this mutant gene is being segregated and transferred into a reassortant in which the other 7 genes are wild type. It should then be possible to determine the effect of this deletion mutation on virulence for monkeys and possibly, man.

It is likely that a range of spontaneous viable deletion mutations occur in other influenza virus genes as well as in the 8th gene itself. Such mutations can now be sought using cell lines persistently expressing a cloned influenza gene. For example, persistent expression of a cloned influenza NP gene has been achieved in a mouse cell line using bovine papilloma virus as a transforming vector; however, it was not possible to transform a significant proportion of these cells. More efficient cell systems for persistent infection can now be constructed using a shuttle vector that incorporates SV40 promoters of transcription and a selectable gene, such as a mutant of dihydrofolate reductase (DHFR) which has a low affinity for methotrexate. In addition to its usefulness in selection of transformed cells, the mutant gene should also facilitate amplification of the recombinant vector when methotrexate is incorporated in the medium.

Our initial efforts to construct influenza DNA recombinants involved the insertion of influenza NP DNA into the DHFR transcription unit between the SV40 promoter and the DHFR coding sequences. Recently, using an immunofluorescence assay it was observed that this recombinant (that codes for a bicistronic mRNA) produced NP in the nuclei of transfected cells. However, the DHFR gene present in the recombinant did not appear to be expressed because selection with methotrexate failed to yield NP producing cells. Because of this observation we constructed recombinants in which each gene was placed under the control of a separate promoter in order to allow the expression of both the inserted influenza DNA and the DHFR gene. In this construction, NP DNA is transcribed using a mouse metallothioneine promoter and SV40 poly A addition sequences. Expression of NP in recombinant DNA transfected CV-1 cells was observed by an indirect immunofluorescence assay. Methotrexate selection of cells is currently in progress. Recombinants of other influenza DNA's, including PB2-DHFR, have been constructed (Ryan, Mackow, Lai).

It should be possible to propagate viruses bearing deletion mutations by using cell lines that persistently express a cloned gene that corresponds to the gene bearing the deletion mutation. Of course, such a cell line must support replication of influenza A virus. For this reason a simian cell line such as CV-1 is being used for this purpose. Cells of this type in which complementation is provided by a wild type influenza protein that is persistently expressed from cloned influenza DNA should provide a permissive system for the efficient growth of viable deletion mutants. Subsequently these mutants can be evaluated for evidence of restriction and attenuation. In this manner, it may be possible to identify naturally occurring genes carrying stable viable deletion mutations that can be used to confer

attenuation on epidemic or pandemic strains of influenza virus by gene reassortment. This approach can also be used to search for deletion mutations in nonsegmented negative strand RNA viruses such as RS virus and the parainfluenza viruses.

Type 3 Parainfluenza Virus

Analysis of type 3 parainfluenza virus with monoclonal antibodies:

Neutralizing monoclonal antibodies to the 1957 prototype parainfluenza type 3 virus were used to map epitopes on the hemagglutinin-neuraminidase (HN) surface glycoprotein and to examine the epidemiology of parainfluenza viruses. Competitive-binding assays indicated that these neutralizing antibodies bind to one antigenic site. Analysis of variants selected in the presence of these antibodies showed that there are 5 distinct epitopes within this area. Examination of parainfluenza type 3 viruses isolated from three geographic regions during the past 25 years showed that antigenic variation occurs but this variation is probably a reflection of genetic heterogeneity among these viruses rather than progressive antigenic drift. Furthermore, all of the variation detected in clinical isolates occurred in only 2 of the 5 defined epitopes on the HN molecule (Van Wyke, Murphy).

Viral Genome: Poly(A) containing RNAs were isolated from cells infected with type 3 parainfluenza virus. Actinomycin D was added to the medium to abolish cellular transcription, and RNAs were resolved by agarose gel electrophoresis under denaturing conditions. Nine major poly A(+) RNA's that were estimated to be 0.3, 0.8, 1.4, 1.5, 1.7, 2.0, 2.5, 3.0, and 7.0 kilobases were visualized. Single stranded ³²P labeled cDNAs were synthesized using mRNA's from infected cells and oligo(dT) as primer. When these cDNAs were analyzed by alkali agarose gel electrophoresis, discrete bands ranging in size from about 300 to 6000 bases were observed. The individual single stranded cDNAs were then made double stranded and cloned in *E. coli* by procedures described previously. In addition, RNA/DNA hybrids were cloned directly after dG/dC tailing. Full length cDNA recombinants large enough to represent DNA copies of individual transcripts were identified by serial colony hybridization. Several recombinants encoding different para-3 transcripts have been identified. Northern blot analysis, hybrid-selection and *in vitro* translation are currently being employed to identify the viral recombinants (Elango, Venkatesan).

Respiratory Syncytial Virus

Cloning and sequence analysis: Human respiratory syncytial (RS) virus, an enveloped non-segmented, negative strand RNA virus, is the major etiologic agent of severe lower respiratory illness of infants and young children. We have used recombinant DNA techniques and biochemical analysis to explore the genome of this virus. A cDNA library representing the poly(A) containing RNA's from RS virus infected cells was constructed in *E. coli* (HB101) using plasmid pBR322 as a vector. The strategy for cloning avoided the traditional approach that includes a self priming reaction for second strand synthesis and subsequent S₁ nuclease treatment to remove the resulting hairpin. We have determined the complete DNA sequences of six RS viral genes cloned in recombinant plasmids. A seventh gene that appears to code for the fusion protein, has also been sequenced; this recombinant lacks about 70 nucleotides corresponding to the 5' end of the message.

It had been anticipated that RS virus would mimic paramyxoviruses and rhabdoviruses in genome organization. However, sequence analysis of several RS viral genes revealed that this is not the case. With rhabdo- and paramyxoviruses, a transcriptional termination signal of four nucleotides (AUAC in the case of VSV and AUUC in the case of Sendai virus) precedes the 7 or 5 U residues that are reiteratively transcribed to form the poly(A) tail. Under certain conditions, the polymerase of these viruses pauses at this signal and generates long poly(A) tails or it may read across the intercistronic region. In influenza viruses, transcription of each segment stops at a cluster of U residues beyond which the genomic sequences are conserved in all segments. However, the individual transcripts that are polyadenylated by a "chattering mechanism" on the U cluster do not exhibit homology upstream of the poly(A) tail. Sequence analysis of seven cloned RS viral genes revealed a similar lack of homology upstream of the poly(A) tail. In contrast, there was conservation of a PuGGGCAAAT....sequence at the 5' end of seven RS viral transcripts. This consensus initiation sequence differs from that found at the 5' end of paramyxovirus and rhabdovirus transcripts. Since this sequence was determined by primer extension, the identity of the initiating nucleotide designated Pu could not be established. However, other data suggests that the initiating nucleotide is probably G in all genes (Venkatesan, Elango, Satake).

Nucleocapsid gene: We were interested in determining the primary structure of RS viral capsid protein in order to define the different domains of this protein that are involved in RNA-protein interactions. In addition, we wished to understand the genetic organization of this human pathogen at the nucleotide level. Our interest in NC protein stems from a desire to understand the nucleocapsid assembly of RS virus and the various interactions between the NC protein and transcriptional enzymes. Knowledge of the primary structure of the major capsid protein should provide insight into the nature of these interactions. To achieve this goal, we have determined the sequence of this protein. A recombinant plasmid (pRSB₁₁) was selected from a RS cDNA library using screening procedures described previously. DNA sequencing of the RS viral insert by Gilbert-Maxam method revealed an RS virus sequence of 1430 nucleotides excluding the poly(A) tail. By primer extension and dideoxy-sequencing the insert was found to lack only four nucleotides corresponding to the 5' end of the mRNA. Of interest, a nine nucleotide sequence 5'-NGGGCAAAT3' was present at the 5' end of the mRNA strand. This sequence is conserved at the 5' end in all seven RS viral genes sequenced so far. The NC clone has a single long open reading frame of 467 amino acids capable of encoding a protein of 51540 daltons. The RS NC sequence did not exhibit homology to VSV, influenza virus, coronavirus and TMV capsid proteins implying that RS virus is quite distinct from these viruses (Elango, Venkatesan).

Matrix protein and phosphoprotein genes: The amino acid sequence of RS viral M protein was deduced from the cDNA sequence of a recombinant plasmid harboring the gene. The RS viral cDNA insert of 950 nucleotides had a poly(A) tail at one end. The other end corresponding to the 5' end of the mRNA lacked five nucleotides (NGGGC) of the mRNA. The cDNA sequence had an open reading frame capable of encoding a protein of 28717 dal (256 amino acids). The protein was relatively basic and moderately hydrophobic. It did not contain regions homologous to other viral matrix proteins. A second open reading frame potentially encoding a protein of 75 amino acids was also

present at the 3' end of the cDNA insert. This overlapped the first reading frame by 20 amino acids. Also several recombinant plasmids containing cDNA encoding the RS viral phosphoprotein (P) gene were identified by a variety of methods. pRSA₃ encoding RS viral P protein was selected for sequencing. It has 916 base pairs of RS viral sequence including a poly A tail of 14 residues. It lacked the NGGG...sequence corresponding to the 5' end of the mRNA. As with other RS viral genes this is part of the conserved sequence 5' NGGGCAAAT. Starting at position 18, there is a single long open reading frame encoding a protein of 241 amino acids with a molecular weight of 27150. It lacks sequence homology with Sendai virus P protein or VSV NS protein which represent counterparts of RS P protein. Unlike the situation reported for the Sendai and measles paramyxoviruses, the RS P gene does not have a second reading frame capable of encoding another protein (Satake, Venkatesan, Elango).

Non-structural protein genes: Recent studies indicate that two RS viral nonstructural proteins (NS1 and NS2) are coded for by two adjacent genes. By means of positive hybrid selection of viral mRNAs and subsequent *in vitro* translation of the selected RNAs a recombinant RS viral plasmid (pRSC₆) that encoded two viral nonstructural proteins was identified. This plasmid contained a RS viral insert of about 1,050 bases and it hybridized to viral mRNAs containing about 500-600 bases as indicated by Northern blot analysis. The insert was sequenced completely and found to contain two different nonoverlapping reading frames that coded for proteins that contained 139 and 124 amino acids respectively. Upstream of the second reading frame there was a nine nucleotide GGGGCAAAT sequence between positions 545-553. The recombinant pRSC₆ was thus shown to be a cDNA clone of a bicistronic transcript. Bicistronic transcripts of RS virus may originate through a failure of the viral polymerase to pause at the intergenic region. Following the polyadenylation sequence of the first gene of the bicistronic transcript (NS1) there is a 19 nucleotide sequence that precedes the 9 nucleotide conserved 5' sequence of the following gene (NS2). This suggests that the 19 base sequence may represent the intergenic region of the RS viral genome. If this intergenic sequence is conserved throughout the genome, RS virus will prove to be even more distinct from the paramyxoviruses and rhabdoviruses than is now appreciated (Venkatesan, Elango).

Glycoprotein genes: RS virus possesses two glycoproteins. One is a 84-88 kdal glycoprotein that lacks hemagglutinin and neuraminidase activities and is referred to as G. The other is a 68 kdal protein that is thought to be the fusion factor (Fo); Fo is proteolytically cleaved into two subunits, F₁ (48 kdal) and F₂ (16 kdal). Since an amino acid sequence for these proteins is not available, we have attempted to deduce this sequence by a combination of recombinant DNA technology and DNA sequencing. A RS viral cDNA insert of 1755 bp hybridized to a viral poly(A) RNA species 2000-2200 bases long. An RNA of this size was shown by Collins and Wertz to be translated *in vitro* to a 59 kdal protein, the putative unglycosylated translation progenitor of the 68 kdal viral fusion glycoprotein. The cDNA insert lacked a poly(A) tail. However, the messenger strand in the insert was established by primer extension of an asymmetrically 5' end labeled restriction fragment on mRNA from infected cells. Dideoxy-sequencing with this primer indicated that the recombinant lacked approximately 70 nucleotides corresponding to the 5' end of the mRNA. pRSA₁₄ has a single long open reading frame, starting at

position 1 and ending at position 1671, that is capable of encoding a partial protein of 556 amino acids. Inspection of the amino acid sequence near the C-terminus revealed a 24 amino acid sequence extremely rich in hydrophobic residues followed by a 25 amino acid sequence rich in hydrophilic and polar residues extending all the way to the C-terminus of the protein. This type of organization is similar to the C-terminus of the influenza A virus hemagglutinin (Elango, Venkatesan, Satake).

Immunity to RS Virus and Its Implications in Vaccination and

Immunotherapy: The cotton rat was used as an experimental animal to test the protective efficacy of passively acquired serum neutralizing antibodies to RS virus (RSV). Infant cotton rats inoculated with varying amounts of homologous convalescent RSV antiserum were subsequently challenged intranasally with RSV. Cotton rats with a circulating titer of neutralizing antibody of 1:350 or greater at the time of challenge were completely resistant to pulmonary infection; cotton rats with a titer of 1:80 to 1:350 became infected but the level of virus replication in the lung was reduced in direct relation to the titer of antibody. In contrast, animals with an antibody titer less than 1:80 sustained an undiminished RSV infection in the lungs. The nose was not protected against RSV even by the highest level of passive serum antibodies. These observations suggest that maternally-derived antibodies can exert a protective effect against RSV infection in the lungs, however, a very high concentration of antibodies is required for significant protection of the lower respiratory tract. The concentration of passive serum antibodies in the circulation of young human infants decreases approximately 50% every month and this may serve to explain the relative sparing of very young infants from serious RSV disease and the markedly increased risk of serious RSV disease during the 2nd, 3rd, 4th, 5th, and 6th months of life. Within a few months after birth the concentration of passively acquired RSV antibodies drops below the critical threshold, 1:80-1:350, and the lungs lose their resistance.

Suspensions of purified human IgG (Sandoglobulin) were shown to contain a very high level of antibodies to RSV. Sandoglobulin administered intraperitoneally to cotton rats prevented pulmonary infection with RSV. More significant, however, was the therapeutic effect of human RSV antibodies administered after infection had become well established. Administration of smaller amounts of Sandoglobulin to owl monkeys which were at the height of their RSV infection also decreased the level of virus in the lungs over the next 2 days. These observations suggest that serious RSV disease in infants may respond to immunotherapy.

In the mid-1960's formalin-inactivated vaccines were prepared against two common enveloped RNA viruses, RSV and measles. Both vaccines were antigenic, but the former did not prove effective in preventing disease, while the latter lost its effectiveness after a few years. In both instances unexpectedly severe illness occurred when vaccinees underwent natural infection. Until now the mechanism of vaccine-induced disease potentiation could not be studied in the laboratory because we lacked a suitable experimental animal. Our recent identification of the cotton rat as a satisfactory experimental model for RSV infection allowed us to examine potentiation induced by an inactivated RSV vaccine in the laboratory.

The original 1962 formalin-killed RSV vaccine ("lot 100") was inoculated intramuscularly into cotton rats, which were subsequently challenged intranasally with live RSV. Within 24 hours a pulmonary cellular infiltrate developed that consisted primarily of polymorphonuclear leukocytes. The infiltrate intensified with time, reaching a maximum at 4 days, the time of peak virus replication in the lungs. The infiltrate then diminished rapidly, eventually resolving without scarring. The histologic appearance of the lesions resembled an experimentally-induced pulmonary Arthus reaction. This pattern of immunopathology was also seen in cotton rats immunized with a newly-prepared suspension of formalin-inactivated RSV. The same type of lesions were also observed in animals which were immunized with formalin-inactivated parainfluenza type 3 virus and subsequently challenged with homologous live virus. Although the lesions in each case resembled an Arthus reaction, we were unable to reproduce the lesions by passive infusion of antibody prior to infection suggesting that the pathogenesis of potentiated disease was more complex than a simple Type III immune reaction.

The essential role of formalin in vaccine-induced altered reactivity to infection was established by comparing formalin-treated (1:4000 formalin, 37°C, 72 hours) and "mock-formalin" (no formalin, 37°C, 72 hours) virus preparations. Both preparations were free of infectious virus but only the formalin-treated virus potentiated disease in the lungs suggesting that the action of formalin on the virus, rather than inactivation per se, was critical to development of lung lesions (Prince, Chanock).

HEPATITIS VIRUSES

Hepatitis B Virus (HBV)

Plasma derived HBs Ag vaccine: Hepatitis B virus (HBV) is a unique 42nm virus of complex structure that contains a double-stranded circular DNA with a single-stranded gap spanning 20-50 percent of the genome. It is the first recognized member of what is now a small group of viruses unofficially designated the "hepadnaviruses". HBV causes considerable morbidity and mortality, accounting for 30-50 percent of clinical hepatitis diagnosed in the U.S. and most developed countries. However, it is in Asia and Africa that the virus has its greatest impact. HBV causes a chronic infection, usually associated with hepatitis, in approximately five percent of the world's population, and this form of HBV infection may lead to death from chronic hepatitis, cirrhosis or hepatic cell carcinoma. Although relatively rare in developed countries, hepatic cell carcinoma is one of the leading causes of death from cancer in Africa and Asia, and there is considerable evidence that HBV is a causative factor in such cancer. For these reasons the control of HBV is an important public health goal.

Research in the Hepatitis Viruses Section as well as in academic and industrial laboratories elsewhere over the past decade has led to the recent development and licensing of hepatitis B vaccines in the United States and France. Such vaccines contain purified HBV surface antigen (HBs Ag) derived from the plasma of individuals chronically infected with HBV. These vaccines have been shown to be both safe and effective in preventing Type B hepatitis.

Clinical testing and characterization of plasma-derived vaccines prepared by the NIH will soon be completed. An alum-adsorbed preparation was found to be highly immunogenic and well tolerated in adult volunteers. Sixty-eight percent of vaccinees developed antibody within one month of vaccination and 95 percent seroconverted following completion of the six-month vaccination schedule. Antibody does appear to persist three to four years or more in some persons (Purcell, Ticehurst).

The rapid antibody response to the NIH vaccine suggested that it may be useful in preventing perinatal transmission of HBV from chronically infected mothers to their offspring, an event that occurs frequently in Asia. In preparation for an efficacy trial of the vaccine in high-risk infants, immunogenicity tests of the NIH vaccine were carried out by Dr. B. Hollinger (Baylor School of Medicine). The NIH vaccine was administered to 65 children 1-17 years old. The NIH vaccine was more immunogenic in young children than in adults: 53 percent of children 11-17 years old developed antibody within two weeks of vaccination, whereas 78 percent of children 1-2 years of age seroconverted by two weeks. By 8 weeks, 100 percent of children 1-10 years of age had developed antibody. Reactions to the vaccine were minimal and not significantly different from those seen in recipients of a placebo preparation. Thus, the NIH vaccine was highly immunogenic and nonreactogenic for children and infants.

An efficacy trial in newborn infants whose mothers are persistently infected with HBV began in the People's Republic of China during the summer of 1982. The NIH vaccine was compared with a placebo in a double-blind randomized study monitored by Dr. Francis of the C.D.C. Drs. Francis and Purcell visited Beijing and Shanghai (where the trials were being conducted) in December of 1983 to evaluate the results obtained with the first 100 infants who had completed the first 6 months of follow-up. The frequency of acquisition of HBV infection by offspring of HBeAg positive mothers was 75 percent by 6 months of age. Despite this frequency of transmission, the NIH vaccine was over 90 percent effective in preventing HBV infection when compared to the experience of placebo recipients. This compares very favorably with the best results obtained by others with HBV vaccine plus hepatitis B immune globulin (HBIG). Final results will not be available until the infants have been followed 1 year (spring-summer of 1984) but the preliminary results are very encouraging and suggest that a potent hepatitis B vaccine, if administered within hours of birth, can prevent most maternal-infant transmission of chronic HBV infection without the need for HBIG (Purcell).

Second and third generation HBV vaccines: Expense, availability and/or incomplete acceptance based upon an unfounded fear of infection with the agent of AIDS limit the impact of plasma-derived hepatitis B vaccines in developed and developing countries. Thus, there is a need for second-generation hepatitis B vaccines that will be readily available, economical and acceptable for wide-spread administration. A number of approaches to the development of such vaccines have been pursued. Most utilize, directly or indirectly, the new recombinant DNA technology. The HBV genome is double-stranded DNA which has four open reading frames that code for four putative proteins, only two of which have been identified (hepatitis B surface antigen, HBsAg, and hepatitis B core antigen, HBcAg).

An intriguing application of recombinant DNA technology to the development of second-generation vaccines was recently described by Dr. B. Moss of LVD. A 1350 bp fragment of HBV DNA that contains the coding sequence for HBsAg was inserted into a vector containing an early promoter of vaccinia virus and the modified vector was used to transfect mammalian cells that were also infected with "wild type" vaccinia virus. Recombination occurred, creating a viable recombinant vaccinia virus that contained the HBV surface antigen gene. Infection of mammalian cells with this recombinant resulted in the synthesis of HBsAg particles. Intradermal infection of rabbits with the recombinant vaccinia virus stimulated the production of antibodies to HBsAg. In collaborative studies with Dr. Moss we observed that chimpanzees similarly infected with the recombinant vaccinia virus did not develop demonstrable HBs antibodies but they were protected against hepatitis when subsequently challenged with live HBV. The immunized chimpanzees developed high titers of anti-HBs at a time when HBsAg was first detected in a control chimpanzee, suggesting that the vaccinated animals had had been "primed" by infection with the recombinant vaccinia and had developed an anamnestic antibody response to newly-synthesized HBsAg following challenge with live HBV. More efficient expression of the HBV surface antigen gene in vaccinia must be engineered before this approach will have practical application in humans (Purcell).

Finally, recombinant DNA technology has permitted not only the molecular cloning of the entire HBV genome but also the determination of its entire nucleotide sequence. The S (surface antigen) gene codes for 226 amino acids that are arranged in a series of alternating hydrophobic and hydrophilic regions. The hydrophilic regions are thought to exist on the surface of the hepatitis B virion (and the HBsAg particle) and the hydrophobic regions probably anchor the polypeptide in the lipid portion of the viral coat. Thus, amino acid sequences in hydrophilic portions probably comprise the antigenic domains that stimulate protective antibody in the host. Peptides consisting of amino acid sequences predicted from the nucleotide sequences of the S gene have been synthesized *in vitro*. Regions representing both the hydrophilic and hydrophobic portions of the HBsAg polypeptide have been studied but the hydrophilic regions appear to be of most interest.

In collaborative studies with Drs. R. Lerner and J. Gerin, synthetic peptides representing a part of one of these hydrophilic regions of the HBsAg polypeptide have been studied intensively. Both linear and cyclic forms (prepared by oxidation of the cysteines to form a disulfide bond) of one of the peptides have been compared. These peptides are similar to synthetic peptides studied by Dreesman et al. A group-reactive specificity (designated as a) as well as a type-specific antigen (the d/y allele) can be found in this region of HBsAg. Polypeptides representing the predicted amino acid sequences for both a subtype adw and a subtype ayw antigen have been synthesized; each stimulates antibody of the appropriate subtype in rabbits. This subtype specificity appears to be an inherent characteristic of the amino acid sequence and may be defined by as few as two amino acid substitutions (at positions 131 and 134), but the group-reactive a specificity is probably steric in nature because it is destroyed by reduction and alkalination of the cyclic peptide. Immunization of chimpanzees with the linear amino acid (aa) 110-137 peptide stimulated anti-HBs that was subtype specific and transient, apparently because it was exclusively of the

IgM class. Nevertheless, these chimpanzees were partially or completely protected when challenged with live HBV. The mechanism of this protection in the absence of demonstrable serum antibody is not known. Additional studies in chimpanzees with linear and cyclic forms of peptide 110-137 and with a short form (aa 125-137) of the peptide that contains the amino acid substitutions defining subtype specificity are currently in progress. Such studies should define the specific sites on the HBsAg molecule that stimulate protective antibody. Synthetic peptides representing one or more of these regions may serve as the basis for the third-generation of hepatitis B vaccines (Purcell).

Woodchuck Hepatitis Virus (WHV)

The woodchuck hepatitis virus (WHV) is taxonomically and serologically related to the hepatitis B virus (HBV). The similarities between HBV and WHV, coupled with the tendency of each virus to cause chronic hepatitis and hepatic cell carcinoma in their respective hosts, makes WHV and its host, the woodchuck, a particularly interesting model system. Sensitive radioimmunoassays for woodchuck hepatitis virus surface antigen (WHsAg) and its antibody (anti-WHs) were developed previously. These permitted an evaluation of a colony of wild-caught woodchucks for markers of infection. Recently a test for anti-WHc was developed and was used to confirm the occurrence of WHV infection in an experimentally inoculated chimpanzee. This infection was markedly attenuated: only anti-WHs and anti-WHc were detected, but these developed in the absence of anti-HBs and anti-HBc, indicating that it was, indeed, WHV that was responsible for the serologic response. Development of antibody to the core antigen, whether HBcAg or WHcAg, is taken to be proof of infection, rather than simple immunization.

A colony of woodchucks was established under contract with Cornell Veterinary School (Dr. B. Tennant, N.Y.S.C.V.M.) to provide facilities for an expanded study of WHV in its natural host. An inactivated vaccine prepared from WHsAg in a manner similar to the hepatitis B vaccine, was administered to selected newborn animals. Other newborn animals received a placebo. Certain of these animals were also inoculated with live WHV at birth and other offspring were exposed under natural conditions to their WHV-positive mothers. The purpose of the study, which will last several years, is to determine whether vaccine can interrupt perinatal transmission of this hepadna virus. This information may allow us to predict the results of similar vaccine interruption trials with HBV vaccine in man. The ultimate goal of these efforts is to prevent human hepatic cell carcinoma (HCC) by preventing initiation of chronic virus infection during the newborn period. However, the efficacy of this approach to prevention of HCC in man will not be known for 20 to 40 years which represents the incubation period from infection to development of HCC. However, the incubation period to HCC in the woodchuck is only 2 to 5 years. Thus, prognostic information should be available from the woodchuck-WHV model system within the next few years. This information should prove to be useful in planning for future use of HBV vaccines in man.

Last year two woodchuck hepatitis virus vaccines were tested and both were found to be safe, (i.e., they did not contain infectious woodchuck virus) and capable of stimulating antibody that protected against challenge with live WHV. The vaccines prevented WHV-associated hepatitis when given

to newborn woodchucks which were simultaneously administered live WHV. In contrast, placebo recipients developed hepatitis when infected with WHV at birth. Although a proportion of the vaccinated woodchucks developed serologic evidence of infection, it was inapparent and detected only by development of anticore antibody. Thus, the woodchuck model predicts that vaccination of the newborn human with hepatitis B vaccine will protect against perinatal transmission of HBV that often results in chronic infection. Surprisingly most of the WHV infections of newborn woodchucks in this study did not become chronic.

Finally, chimpanzees and woodchucks were vaccinated with WHV vaccine and HBV vaccine respectively. The vaccinated animals were then challenged with the heterologous virus (HBV in chimpanzees and WHV in woodchucks). This study was made possible by the previous demonstration that each of the two vaccines was capable of protecting against its homologous virus. The purpose of the cross protection study was to determine if antigens shared by the two viruses reflect a conserved region of their genomes that might code for a function essential to initiation of infection, such as attachment to hepatocytes. Identification of an important protective site (or sites) would be achieved if a shared antigenic domain (or domains) was shown to elicit cross protection. Preliminary observations indicate that chimpanzees vaccinated with WHV vaccine were protected from HBV infection but woodchucks vaccinated with HBV vaccine were not protected from WHV infection, thus confirming the existence of a one-way serological cross-reaction between the two viruses (Purcell).

Hepatitis A Virus (HAV)

Cultivation and attenuation of HAV: The hepatitis A virus (HAV) is a picornavirus that was isolated in tissue culture for the first time relatively recently. Several laboratories, including the Hepatitis Viruses Section, have succeeded in confirming the isolation of HAV. In addition, we have succeeded in isolating virus directly from human clinical materials without the intervening serial passage in marmoset monkeys that was necessary for the initial isolation in tissue culture. We have used as cell substrate primary African green monkey kidney cells, a cell type approved for human vaccines.

More than 30 serial passages of HAV in primary African green monkey cells have been achieved. Initially, 8 or more weeks were required to demonstrate adequate replication of the virus; however, this can now be achieved in 1-4 weeks. Most of the viral antigen remains cell-associated. The infectivity titer of HAV in tissue culture following 10 passages was approximately 10^6 per ml of cell lysate. By passage 16, this had increased to 10^8 .

The tissue culture-adapted virus became attenuated when compared to the parent virus, which produces evidence of liver damage in chimpanzees. Tissue culture-adapted virus from the 10th and 20th passages produced infection that was followed by a brisk antibody response, but there was little or no histological or biochemical evidence of liver disease. The stability of the attenuated tissue culture-passaged virus was evaluated by inoculating additional chimpanzees with acute phase stools of chimpanzees infected with attenuated HAV. The virus produced a seroconversion in the

recipient chimpanzees but there was no biochemical evidence of liver disease during 3 serial passages in chimpanzees. Most chimpanzees which had previously been infected with the attenuated virus (P-10, P-20) were protected from hepatitis when challenged with virulent strain. In contrast to the response of chimpanzees, marmosets inoculated with cell culture P-20 developed significant enzyme elevations. Thus, attenuation of HAV was species specific.

Tissue culture-adapted HAV has been cloned by terminal dilution at 3 passage levels - 10, 20, and 30. Recently, two clones of triply cloned master seed (P-20) were evaluated for evidence of attenuation in chimpanzees and marmosets. This virus infected chimpanzees and induced a seroresponse; however, enzyme elevations were detected in 2 of the 8 inoculated animals. In addition, marmosets inoculated with one of the clones developed enzyme elevations. These findings indicate further attenuation of the cloned virus may be necessary. Triply cloned virus at the 30th passage is currently being tested for attenuation (Feinstone, Purcell).

Cloning and sequence analysis of HAV genome: Initially efforts at cloning HAV cDNA were impeded by the small amount of virus available and the instability of virion RNA extracted from purified virus. Once these obstacles were overcome double-stranded cDNA fragments were synthesized from hepatitis A virus (HAV) RNA and inserted into the Pst I site of pBR322. The identity of cloned cDNA was established by demonstrating its hybridization to RNA from HAV-infected tissue culture cells but not to RNA from uninfected cells. Genomic length RNA of approximately 7500 nucleotides was the predominant species that hybridized with the cloned HAV cDNA. Restriction endonuclease analysis and hybridization between subgenomic fragments yielded a map of overlapping cloned cDNAs which included 99% of the viral genome. The extent of RNA sequence not represented in cDNA clones was determined by primer extension on RNA from HAV-infected cells. The estimate that cloned cDNA extended to within 30 nucleotides of the 5' end of the genome represented independent confirmation of the size and orientation of the restriction map (Ticehurst, Baroudy, Feinstone, Purcell).

A sequence of 3119 bases corresponding to the 5' end of HAV RNA was determined. This sequence contains an open reading frame that begins \approx 750 bases from the 5' terminus and extends 2407 bases which is as far as sequence has been determined. Sequence preceding this major reading frame has nine other potential initiation sites, but the longest peptide that can be translated in this region is only 26 amino acids. This pattern is consistent with the genomic organization of other picornaviruses. The entire sequence of a clone that mapped to the 3' end of HAV RNA was also determined. A poly(A) tract of 15 bases was found at one end of this clone thus orienting the 3' end of the genome. This poly (A) tract is 51 bases downstream from two closely spaced termination codons that are preceded by 1407 bases in an open reading frame that is presumably continuous with that present at the 5' end of the genome (Baroudy, Ticehurst).

The sequences of the two translated regions of the HAV genome have been compared to analogous regions of other previously sequenced picornaviruses. A number of amino acid homology programs for computer analysis have been used for these studies, in collaboration with Dr. Maizel. The most extensive relatedness was found in the 3' area that codes for RNA polymerase

of poliovirus. This domain is conserved in all of the genomic sequences that have been examined, that is, HAV, poliovirus, foot and mouth disease virus (FMDV), and rhinovirus. Dot matrix analysis indicates that the relatedness of the HAV 3' polypeptide to poliovirus RNA polymerase is less than that of rhinovirus, but more than that of FMDV (Ticehurst, Baroudy, Feinstone, Purcell).

Hybridization has also been used for detection of HAV RNA in tissue culture and fecal specimens. Suspensions were treated with protease, detergent, and organic solvents to release any HAV RNA present. Nucleic acid preparations were then applied to nitrocellulose (directly or by suction through a filter manifold) and hybridized with nick translated cloned cDNA. This type of hybridization was more sensitive for detection of HAV than radioimmunoassay of HAV proteins (Ticehurst, Purcell, Feinstone).

HAV monoclonal antibodies: Production and characterization of monoclonal antibodies to hepatitis A virus is in progress. The development of new serologic tests for detection of monoclonal anti-HAV has simplified the procedure. Five specific monoclonal antibodies have been produced and are being analyzed (Feinstone).

Delta agent

The delta agent is a transmissible hepatitis agent that appears to be defective; it requires co-infection with hepatitis B virus for its own synthesis. The agent has a small RNA genome ($10^{5.7}$ daltons) that is encapsidated together with delta antigen within a coat of HBs Ag. The agent was discovered in 1977 in Italy, where it is endemic. Evidence for infection with the delta agent is found most frequently in carriers of hepatitis B virus who are repeatedly exposed to blood (hemophiliacs, illicit drug users, etc.). Sensitive assays for delta agent infection have been developed and used to evaluate experimental delta infection of HBV-carrier chimpanzees. In both man and chimpanzee infection with the delta agent results in very severe hepatitis. The delta agent has also been experimentally transmitted to woodchucks chronically infected with the woodchuck hepatitis virus, a virus similar to hepatitis B virus. The chimpanzee and woodchuck provide animal model systems for more detailed characterization of this medically important group of agents.

There are analogies between the delta agent and certain pathogens of plants, namely, the plant virus satellites. These phytopathogens a) have RNA genomes that, in some cases, are comparable in size to that of the delta agent, b) are defective, requiring a helper function from non-defective viruses, c) are encapsidated with the structural proteins of the helper virus and, d) in some cases potentiate disease caused by the helper virus. However, unlike the plant viruses and their satellites, which both contain RNA, the RNA-containing delta agent requires coinfection with a DNA-containing hepadnavirus.

Recently, in a collaborative study with Dr. S. Hadler (CDC), Dr. Antonio Ponzetto (Expert Consultant to LID from Turin, Italy) obtained serologic evidence for an etiological association between the delta agent and severe hepatitis with high mortality in Yuca Indians living in western Venezuela. Previous epidemiologic evaluation of this very severe Venezuelan

hepatitis had suggested an association with HBV infection, but a high prevalence of serologic markers of HBV infection in non-affected villagers made the association tenuous. However, when sera were tested for evidence of delta infection, virtually every patient who had experienced the severe form of hepatitis was found to have evidence of infection with the delta agent. In contrast, virtually none of the nonaffected HBV-positive individuals in other villages had evidence of delta infection. Death from this form of Venezuelan hepatitis resulted from either fulminant hepatitis or a rapidly progressive form of subacute and chronic active hepatitis (Purcell).

Other Non A, Non B Hepatitis Viruses

Epidemic non-A, non-B hepatitis: New hepatitis agents continue to be recognized. Recently, a form of epidemic hepatitis occurring in India could not be associated with any of the recognized hepatitis viruses. Attempts to transmit an agent from acute-phase clinical samples to primates are in progress and these efforts have been partially successful. Transient low-level liver enzyme elevations and histopathologic changes consistent with hepatitis in liver biopsies have been observed in some animals, but this has not been uniform, and attempts to serially transmit an agent in chimpanzees and marmosets have also produced irregular results. Characteristic histopathologic changes distinct from those seen in type A hepatitis, type B hepatitis and non-A non-B hepatitis have been reported by Dr. Hans Popper (Mt. Sinai, New York), (Purcell).

Sporadic non-A, non-B hepatitis: As serologic tests for HAV and HBV infection are more widely applied, it is becoming apparent that non-A, non-B hepatitis agents are widely distributed and associated with a significant proportion of clinical hepatitis, perhaps 25 percent of all clinical cases and as much as 90 percent of post-transfusion hepatitis. A number of these agents have been successfully transmitted to chimpanzees, but despite extensive efforts, an antigen-antibody system unequivocally specific for non-A, non-B infections has not been detected. An exception is delta antigen, an antigen associated with a unique non-A, non-B agent that is defective and requires coinfection with hepatitis B virus for its own synthesis. The lack of a serologic test for non-A, non-B agents has made control of these pathogens extremely difficult.

Although non-A, non-B hepatitis agents cannot be detected by serologic means, they can be experimentally transmitted to chimpanzees and marmosets. These species have been useful in determining the infectivity titers of various plasmas that contain non-A, non-B virus. Although most plasmas contain only 10^2 - 10^3 infectious units per ml, one plasma was found to contain over 10^6 infectious units per ml. This plasma has provided an inoculum suitable for characterization of the agent. We have recently demonstrated that at least this one non-A, non-B agent contains essential lipids. This property should prove to be useful in the classification and control of non-A, non-B hepatitis (Feinstone, Purcell).

Collaborative studies designed to develop methods for inactivation of non-A, non-B hepatitis viruses in blood products are underway. Incubation of lyophilized anti-hemophilic factor (AHF) at 60°C for 30 hours inactivated one strain of non-A, non-B hepatitis virus. This virus was also inactivated

by extraction of AHF with chloroform. These treatments show some promise for commercial application because the potency of AHF was not affected (Purcell, Feinstone).

Molecular Biology of Dengue Virus (A Flavivirus)

Dengue viruses are members of the flavivirus group of togaviridae that contain a positive strand RNA genome of approximately 10-12 kilobases. We employed recombinant DNA techniques to investigate the molecular biology of dengue virus with the intent of developing immunoprophylactic measures against this virus group that is epidemic in many geographical areas. The 42S full-length RNA from dengue virus type 4, produced in C6/36 mosquito cells, was isolated and tailed with poly(A) at the 3'-terminus using E. coli poly(A) polymerase. Complementary DNA was synthesized by reverse-transcription using oligo(dT) as a primer and subsequently converted to double stranded DNA by oligo d(C) tailing and oligo d(G) priming. The dengue DNA product was inserted into the Pst I site of pBR322 using dG/dC linker sequences. E. coli transformants containing dengue virus specific sequences were identified by in situ colony hybridization. Characterization of recombinant plasmids from several transformants showed that these DNA inserts were short in length ranging from 200 to 300 base pairs. Nevertheless, the dengue specific sequences should prove useful for reverse-transcription extension of virion RNA or replicative form RNA in order to clone longer, and ultimately full-length cDNA copies of the dengue genomic RNA sequence. Cloned dengue virus DNA will be used for: (1) mapping and sequencing of viral genes; (2) synthesis of viral polypeptides; and (3) construction of full-length infectious dengue virus DNA (Zhao, Lai).

Honors and Awards

Robert Chanock

Elected to Council of American Society for Virology 1984.

Invited speaker: Society for General Microbiology Symposium: Mechanisms of Pathogenicity of Respiratory Tract Viruses, Univ. of Reading, UK, January 1984.

Co-organizer and co-chairman of Second Cold Spring Harbor Conference on Modern Approaches to Vaccines, Cold Spring Harbor, NY, September 1984.

Invited speaker, London Colloquium on Influenza Celebrating 50 Years of Research on the Influenza Viruses, London, UK, September 1983.

Co-chairman of Symposium "New Concepts in Immunization, Sixth International Congress of Virology, Sendai, Japan, September 1984.

Co-organizer of Fogarty Center Conference on New Approaches to Control of Viral Infections, NIH, Bethesda, MD, June 1984.

Albert Kapikian

Invited to attend 7th Meeting of the Steering Committee of the Scientific Working Group on Viral Diarrheas of the Diarrheal Diseases Central Programme of WHO, Geneva, Switzerland, July 1983.

Invited speaker, Second Group Meeting of the European Group for Rapid Viral Diagnosis, "Immune Electronmicroscopy in Virology", Turku, Finland, August 1983.

Invited to attend Seventeenth Joint Working Conference on Viral Diseases, US-Japan Cooperative Medical Science Program. Co-chairman of session on Viral Gastroenteritis, San Diego, CA, September 1983.

Invited speaker, 14th Annual Meeting of Japanese Pediatric Association for Viral Diseases, Sendai, Japan, December 1983.

Invited to attend 8th Meeting of the Steering Committee of the Scientific Working Group on Viral Diarrheas of the Diarrheal Diseases Central Programme of WHO, Geneva, Switzerland, January 1984.

Invited to attend and make presentation, 3rd Meeting of Scientific Working Group on Microbiology, Immunology, and Vaccine Development in Viral Diarrheas, Geneva, Switzerland, February 1984.

Invited speaker, Viral Gastroenteritis, Merck, Sharpe, and Dohme Research Laboratories, West Point, PA, March 1984.

Ching-Juh Lai

Organizer and speaker; New Approaches to Control of Viral Infections, AIT-CCNAA Joint Seminar, Fogarty International Center, NIH, Bethesda, MD, June 1984.

Invited speaker, Symposium on the molecular biology of neoplasia, Taipei, Japan, June 1984.

Brian Murphy

Invited speaker, Cold Spring Harbor Symposium on Modern Approaches to Vaccines, Cold Spring Harbor, NY, September 1984.

Invited lecturer, St. Jude Children's Hospital, Memphis, TN, January 1984.

Invited lecturer, Mount Sinai School of Medicine, Dept. of Microbiology, New York, NY, January 1983.

Invited speaker, AIT-CCNAA symposium on "New Approaches to Control of Viral Infections", Fogarty International Center, NIH, Bethesda, MD, June 1984.

Robert Purcell

Invited speaker, Fourth International Symposium on Viral Hepatitis, San Francisco, CA, March 1984.

Invited speaker, Symposium on New Approaches to Control of Viral Infections, Fogarty International Center, NIH, Bethesda, MD, June 1984.

Invited speaker, Symposium on New Hepatitis B vaccines and clinical studies needed for licensures, Bethesda, MD, June 1984.

Elected to International Association for the Study and Prevention of Virus Associated Cancers.

Bahige Baroudy

Invited speaker, Second Cold Spring Harbor Conference on Modern Approaches to Vaccines, Cold Spring Harbor, NY, September 1984.

Stephen Feinstone

Invited Participant, International Symposium on Viral Hepatitis, Turin, Italy, June 1983.

Editorial Board of The Journal of Hygiene, 1983.

Guest Faculty, Walter Reed Army Institute of Research, Tropical Medicine Course, 1981, 1982, 1983.

Jorge Flores

Invited speaker, Central American Society of Microbiology Conference,
Guatemala, December 1983.

Invited speaker, Pediatric Infectious Diseases Society Conference, Chile,
July 1984.

Invited speaker, Cold Spring Harbor Conference on Modern Approaches to
Vaccines, Cold Spring Harbor, NY, September 1983.

Invited speaker, Seventeenth Joint Working Conference on Viral Diseases,
US-Japan Cooperative Medical Science Program, San Diego, CA, September
1983.

Invited speaker, "New and Useful Methods in Rapid Viral Diagnosis",
Bethesda, MD, September 1983.

Lewis Markoff

Invited speaker, Fogarty Center Conference on New Approaches to Control of
Viral Infections, NIH, Bethesda, MD, June 1984.

Invited assistant rapporteur, International Symposium on Poliomyelitis
Control, Pan American Health Organization, Washington, D.C., March
1983.

Mark Snyder

Invited speaker, American Society of Virology Symposium, Madison, WI, June
1984.

John Ticehurst

Selected speaker, Fourth International Symposium on Viral Hepatitis, San
Francisco, CA, March 1984.

Richard Wyatt

Invited speaker, Control and Eradication of Infectious Diseases in Latin
America, San Jose, Costa Rica, February 1984.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00308-03 LID

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

In Vitro Studies of Hepatitis A Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---------|----------------------------|------------------|------------|
| PI: | Robert H. Purcell, M.D. | Head, HV Section | LID, NIAID |
| Others: | Stephen M. Feinstone, M.D. | Medical Officer | LID, NIAID |
| | Richard J. Daemer, Ph.D. | Microbiologist | LID, NIAID |

COOPERATING UNITS (if any)

Smith-Kline-RIT (Dr. d'Hondt)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

0.6

PROFESSIONAL:

0.3

OTHER

0.3

CHECK APPROPRIATE BOX(ES)

- | | | |
|---|--|---|
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input checked="" type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors | | |
| <input type="checkbox"/> (a2) Interviews | | |

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Hepatitis A virus has been successfully adapted to growth in African green monkey kidney tissue culture. Over 28 serial passages have been achieved, with infectivity titers as high as 10^8 infectious units per ml of cell concentrate. The virus is predominantly cell-associated and does not produce cytopathic effects (CPE). It was attenuated for chimpanzees after 10 serial tissue culture passages; reevaluation of the virus in chimpanzees after 20 tissue culture passages indicates the virus infectivity and attenuation have remained the same as at passage level 10. The attenuated virus has been shown not to revert to virulence during serial passage through chimpanzees. Immunization of chimpanzees with the attenuated virus is protective. The tissue culture-adapted HAV is being 3X cloned. Two clones have been characterized in chimpanzees and marmosets.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00309-03 LID

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Biology of Hepatitis A Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---------|----------------------------|----------------------|------------|
| PI: | Stephen M. Feinstone, M.D. | Medical Officer | LID, NIAID |
| Others: | Richard J. Daemer, Ph.D. | Microbiologist | LID, NIAID |
| | Robert H. Purcell, M.D. | Head, HV Sect. | LID, NIAID |
| | John Ticehurst, M.D. | Medical Staff Fellow | LID, NIAID |
| | Bahige Baroudy, Ph.D. | Visiting Associate | LID, NIAID |
| | Hanson Koo, M.D. | Visiting Fellow | LID, NIAID |

COOPERATING UNITS (if any)

Fairfield Hospital, Melbourne, Australia (Dr. I. Gust)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

0.9

OTHER:

0.6

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have successfully isolated a strain of hepatitis A virus in African green monkey kidney tissue culture, a cell substrate suitable for vaccine development. Growth of the agent in vitro has been characterized and attenuation for chimpanzees documented. The strain of virus appears to have considerable potential for vaccine development.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00310-03 LID

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Hybridoma Antibodies to Pathogenic Viruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Stephen M. Feinstone, M.D. Medical Officer LID, NIAID
Others: Jingsing Mao, M.D. Visiting Fellow LID, NIAID
Robert H. Purcell, M.D. Head, HV Sect. LID, NIAID

COOPERATING UNITS (if any)

NIAID, NIH, Bethesda, Maryland (Dr. A. Fauci)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

1.0

PROFESSIONAL

1.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Production and characterization of monoclonal antibodies to hepatitis A virus is in progress. The development of new serologic tests for detection of monoclonal anti-HAV has simplified the procedure. Five specific monoclonal antibodies have been produced and are being analyzed.

| | | |
|---|--|--|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00311-03 LID |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Search for New Hepatitis Agents | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) | | |
| PI: Robert H. Purcell, M.D. Others: Kendo Kiyosawa, M.D. Albert Kapikian, M.D. John Ticehurst, M.D. | Head, HV Section Visiting Associate Head, Epidemiology Sect. Medical Staff Fellow | LID, NIAID LID, NIAID LID, NIAID LID, NIAID |
| COOPERATING UNITS (if any) Clin. Center Blood Bank, NIH, Bethesda, MD (Dr. Alter); Nat. Inst. Virol., Pune, India (Dr. Pavri); Med. College Srinagar, India (Dr. Khuroo); Mt. Sinai Hosp., NYC (Dr. Popper); Inst. Pasteur d'Algerie, Algiers, (Dr. Belsbbs) | | |
| LAB/BRANCH Laboratory of Infectious Diseases | | |
| SECTION Hepatitis Viruses Section | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MAN-YEARS: 0.9 | PROFESSIONAL: 0.5 | OTHER: 0.4 |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) New hepatitis agents continue to be recognized. Recently, a form of epidemic hepatitis occurring in India was found not to be caused by recognized hepatitis viruses. Attempts to transmit an agent from acute-phase clinical samples to primates are in progress. Attempts to transmit an agent from clinical specimens from these outbreaks have been partially successful. Transient low-level liver enzyme elevations and histopathologic changes consistent with hepatitis in liver biopsies have been observed in some animals, but this has not been uniform, and attempts to serially transmit an agent in chimpanzees and marmosets have also produced irregular results. Characteristic histopathologic changes distinct from those seen in type A hepatitis, type B hepatitis and non-A non-B hepatitis have been reported by Dr. Hans Popper (Mt. Sinai, New York). | | |

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00312-03 LID

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical and Experimental Studies of Hepatitis B Vaccines

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: John Ticehurst, M.D. Medical Staff Fellow LID, NIAID

Others: Robert H. Purcell, M.D. Head, HV Sect. LID, NIAID

COOPERATING UNITS (if any)

Baylor School Med., Houston, TX (Dr. Hollinger); Div. Mol. Virol. & Immunol., G.U., Wash. DC (Dr. Gerin); Dept. Med., Rutgers Med. School, New Brunswick, NJ (Dr. McAuliffe); CDC, Atlanta, GA (Dr. Francis).

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

0.3

PROFESSIONAL:

0.2

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Clinical testing and characterization of plasma-derived hepatitis B vaccine prepared by the NIAID will soon be completed. The vaccine is highly immunogenic, safe and well tolerated when tested in healthy persons ranging from infants to adults. Preliminary results from an efficacy trial in Asia suggest that the vaccine effectively prevents transmission of hepatitis B virus infection from mothers to infants.

| | | |
|--|---|---|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER <div style="border: 1px solid black; padding: 2px; text-align: center;">Z01 AI 00313-03 LID</div> |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular Biology of Hepatocellular Carcinoma | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) | | |
| PI: Robert H. Purcell, M.D. | Head, HV Section | LID, NIAID |
| Others: Richard J. Daemer, Ph.D. Kendo Kiyosawa, M.D. He Li-Fang, M.D. | Microbiologist Visiting Associate Visiting Fellow | LID, NIAID LID, NIAID LID, NIAID |
| COOPERATING UNITS (if any) | | |
| LAB/BRANCH Laboratory of Infectious Diseases | | |
| SECTION Hepatitis Viruses Section | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MAN-YEARS: 1.0 | PROFESSIONAL: 0.9 | OTHER: 0.1 |
| CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div> | | |
| SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) <p> Many tumor-bearing animals develop antibodies to unique antigens associated with the oncogenic virus causing the tumor. These antigens, called "neoantigens," have been found in tumors caused by papovaviruses, adenoviruses, and herpes viruses. Hepatitis B virus, a hepadnavirus with suspected oncogenic potential, cannot be transmitted to non-primates but patients with HBV-associated hepatoma might be expected to have antibody to a HBV-associated neoantigen if one exists. Using a hepatoma cell line that contains integrated HBV DNA, we sought immunofluorescent antibody in sera of hepatoma patients. Approximately seven percent of sera from HBsAg-positive hepatoma patients contained an antibody that reacted with a nuclear antigen in the hepatoma cell line. This antigen was found in another hepatoma cell line that also contained integrated HBV genome but not in two other hepatoma cell lines lacking HBV genome. The antigen ("hepatitis B virus-associated nuclear antigen": HBNA) is being further characterized to determine if it is the product of a transforming gene. A different nuclear antigen was found in a human hepatoma cell line that did not contain HBV DNA. It was identified with serum from a patient with HBsAg negative hepatocellular carcinoma. The new antigen has characteristics similar to those of HBNA. </p> | | |

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|--|---------------------|---------------------------------------|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00314-03 LID |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Woodchuck Virus | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) | | |
| PI: Robert Purcell, M.D. | Head, HV Section | LID, NIAID |
| COOPERATING UNITS (if any) Div. Molec. Virol. & Immunol., Georgetown U, Washington, DC (Dr. Gerin); New York State College of Veterinary Medicine (Dr. B. Tennant) | | |
| LAB/BRANCH Laboratory of Infectious Diseases | | |
| SECTION Hepatitis Viruses Section | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda Maryland 20205 | | |
| TOTAL MAN-YEARS 0.2 | PROFESSIONAL 0.1 | OTHER 0.1 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The woodchuck hepatitis virus (WHV) is taxonomically and serologically related to the hepatitis B virus (HBV). Infection with each of these viruses is associated with acute and chronic hepatitis and hepatic cell carcinoma in their respective hosts; these associations appear to be etiological in nature. The WHV/woodchuck model system provides a convenient means of studying the relationship between virus and host in the oncogenic process. Tests specific for the WHV antigen-antibody systems have been developed. They are being applied to a prospective study of WHV infection in newborn woodchucks and the ability of active immunization to prevent hepatic cell carcinoma. The results of the study should have important prognostic value in evaluating active immunoprophylaxis of hepatitis B virus in man. Preliminary observations indicate that WHV vaccine protects chimpanzees against HBV infection, however, HBV vaccine does not protect woodchucks against WHV infection. </p> | | |

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00315-03 LID

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Search for New Hepadnaviruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: John Ticehurst, M.D. Medical Staff Fellow LID, NIAID

Others: Robert H. Purcell, M.D. Head, HV Sect. LID, NIAID
Diane Blackmore CO-STEP LID, NIAID

COOPERATING UNITS (if any)

Veterinary Resources Branch, NIH (R. Whitney); Div. Molecular Virology, Georgetown U., Washington, D.C. (P. Cote)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

0.1

PROFESSIONAL:

0.1

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Viruses similar to hepatitis B virus ("hepadnaviruses") have been identified in three non-human species: the eastern woodchuck, the Beechey ground squirrel and the Pekin duck. It is likely that many other species harbor similar viruses. The existing animal hosts are of limited value because inbred strains suitable for detailed immunological studies are not available. An attempt was made to transmit woodchuck hepatitis virus to cotton rats and guinea pigs but we could not detect evidence of infection. We are also searching for hepadnaviruses among inbred strains of rodents, especially those with a known high incidence of hepatoma, in hopes of finding a more useful animal model system. Sensitive assays of hepadnavirus infection have been modified to permit testing of the small quantities of serum available.

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| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00316-03 LID |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) The Delta Agent | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) | | |
| PI: Robert H. Purcell, M.D. | Head, HV Section | LID, NIAID |
| COOPERATING UNITS (if any) Georgetown U., Washington, DC (Dr. Gerin); CDC, Phoenix, Arizona (Dr. Hadler); Sinai Hospital, NYC (Dr. Popper); Chiba U., Chiba City, Japan (Dr. Omata); U. Washington Med. Research Unit, Taipei, Taiwan (Dr. Bessley) | | |
| LAB/BRANCH Laboratory of Infectious Diseases | | |
| SECTION Hepatitis Viruses Section | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MAN-YEARS <div style="text-align: center;">0.4</div> | PROFESSIONAL <div style="text-align: center;">0.1</div> | OTHER: <div style="text-align: center;">0.3</div> |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The delta agent is a transmissible hepatitis agent that appears to be defective and requires co-infection with hepatitis B virus for its own synthesis. The agent has a small RNA genome ($10^{5.7}$ daltons) that is encapsidated together with delta antigen within a coat of HBs Ag. The agent was discovered in 1977 in Italy, where it is endemic. Evidence for infection with the delta agent is found most frequently in carriers of hepatitis B virus who are repeatedly exposed to blood (hemophiliacs, illicit drug users, etc.). Sensitive assays for delta agent infection have been developed and used to evaluate experimental infection of HBV-carrier chimpanzees. In both man and chimpanzee infection with the delta agent results in very severe hepatitis. The delta agent has been experimentally transmitted to woodchucks chronically infected with the woodchuck hepatitis virus, a hepatitis virus similar to hepatitis B virus. The chimpanzee and woodchuck provide animal model systems for more detailed characterization of this medically important agent. Recently, serologic studies of Yucpa Indians (Venezuela) indicated that delta agent was etiologically associated with severe and often fatal hepatitis in this population which has a high incidence of hepatitis B virus infection. </p> | | |

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00317-03 LID

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biology of Non-A, Non-B Hepatitis Agents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Stephen M. Feinstone, M.D. Medical Officer LID, NIAID

Others: Robert H. Purcell, M.D. Head, HV Section LID, NIAID

He Li Fang, M.D. Visiting Fellow LID, NIAID

COOPERATING UNITS (if any)

Blood Bank, NIH Clinical Center, Bethesda, MD (Dr. Alter, Dr. Shiraishi); CH, LMG, NIH, Bethesda, MD (Dr. Dawid, Dr. Sargent);

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.0

PROFESSIONAL:

0.8

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Although non-A, non-B hepatitis agents cannot be detected by serologic means, they can be experimentally transmitted to chimpanzees and marmosets. These species have been useful in determining the infectivity titers of various plasmas that contain non-A, non-B virus. Although most plasmas contain only 10^2 - 10^3 infectious units per ml, one plasma was found to contain over 10^6 infectious units per ml. This plasma has provided an inoculum suitable for characterization of the agent. We have recently demonstrated that at least one non-A, non-B agent contains essential lipids, a characteristic that will be important in the classification and, probably, the control of non-A, non-B hepatitis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00318-03 LID

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Molecular Biology of Non-A, Non-B Hepatitis Agents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Robert H. Purcell, M.D. Head, HV Section LID, NIAID
Others: Stephen M. Feinstone, M.D. Medical Officer LID, NIAID

COOPERATING UNITS (if any)

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Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

0.4

PROFESSIONAL

0.1

OTHER

0.3

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Attempts to identify non-A, non-B hepatitis agents by serologic means have been uniformly unsuccessful throughout the world. We have sought to apply recent advances in nucleic acid chemistry to a search for the genome of the non-A, non-B agent. Sensitive radiolabeling procedures have been modified to permit labeling of minute quantities of nucleic acid. Identification of the genome of the non-A, non-B agent would permit its characterization and cloning.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00319-03 LID

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

New Approaches to the In Vitro Propagation of Non-Cultivable Agents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Robert H. Purcell, M.D. Head, HV Section LID, NIAID

Others: Richard J. Daemer, Ph.D. Microbiologist LID, NIAID

COOPERATING UNITS (if any)

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Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

0.5

PROFESSIONAL:

0.2

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

There is a need for an in vitro substrate for the cultivation of hepatitis viruses. Hepatocytes would seem a logical choice, but it is very difficult to obtain and maintain primate hepatocytes in culture. We are attempting to develop hepatocyte-hepatoma hybridomas of primate origin. Such hybrid cells would be expected to have the receptor sites and metabolic systems suitable for synthesis of hepatitis viruses and the ability of hepatoma cells to multiply indefinitely in vitro.

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| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER ZOI AI 00320-02 LID |
| PERIOD COVERED October 1, 1982 through September 30, 1983 | | TERMINATED |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Parvovirus-like Agents and Hematological Disorders | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) | | |
| PI: Robert H. Purcell, M.D. Head, HV Section | LID NIAID | |
| Others: Philip Mortimer, M.D. Guest Worker | LID NIAID | |
| COOPERATING UNITS (if any) NHLBI (Dr. H. Young); Children's Hospital, Washington, D.C. (Dr. Rodriguez). | | |
| LAB/BRANCH Laboratory of Infectious Diseases | | |
| SECTION Hepatitis Viruses Section | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MAN-YEARS <div style="text-align: right;">0.2</div> | PROFESSIONAL <div style="text-align: right;">0.1</div> | OTHER: <div style="text-align: right;">0.1</div> |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Severe hypoplastic crisis among patients with sickle cell anemia has been associated with infection with what appears to be a non-cultivable parvovirus-like agent. Immune adherence hemagglutination and radioimmunoassay techniques have been adapted to identification of this agent and its antibody: approximately 60 percent of normal populations have been found to be antibody-positive. Attempts to transmit the serum parvovirus-like agent to primates are in progress. Attempts to demonstrate replication in tissue culture have been partially successful.</p> <p style="text-align: center; margin-top: 20px;">T E R M I N A T E D</p> | | |

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00321-03 LID

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development, Characterization, and Use of Cloned Hepatitis A Virus cDNA

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---------|----------------------------|----------------------|------------|
| PI: | John Ticehurst, M.D. | Medical Staff Fellow | LID, NIAID |
| Others: | Stephen M. Feinstone, M.D. | Medical Officer | LID, NIAID |
| | Robert H. Purcell, M.D. | Head, HV Sect. | LID, NIAID |
| | Bahige M. Baroudy, Ph.D | Visiting Associate | LID, NIAID |
| | Nickolaos Tassopoulos | Visiting Scientist | LID, NIAID |

COOPERATING UNITS (if any)

Columbia U., NY (Dr. Racaniello); MIT, Cambridge, Mass.
(Dr. D. Baltimore); Chiron Corp. (Dr. Dina); UCSF (Dr. Rutter)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

3.3

PROFESSIONAL:

1.6

OTHER:

1.7

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Double-stranded cDNA fragments were synthesized from hepatitis A virus (HAV) RNA and inserted into the Pst I site of pBR322. The identity of cloned cDNA was established by demonstrating its hybridization to RNA from HAV-infected tissue culture cells but not to RNA from uninfected cells. Genomic length RNA of approximately 7500 nucleotides was the predominant species that hybridized with the HAV cloned HAV cDNA. Restriction endonuclease digestion and hybridization between subgenomic fragments yielded a map of overlapping cloned cDNAs which included 99% of the viral genome. Cloned cDNA from near the 5' terminus of the genome was used to synthesize and clone cDNA by primer extension so that molecular clones could be obtained that contained the 5' terminus of the genome. Cloned cDNA was used as a probe for detecting HAV RNA in tissue culture and fecal specimens by hybridization.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00370-02 LID

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)
Studies of Acquired Immune Deficiency syndrome (AIDS)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Robert H. Purcell, M.D. Head, HV Section LID, NIAID

COOPERATING UNITS (if any)

NINCDS, NIH, Bethesda, MD (Dr. London); New York Blood Center, NYC, NY (Dr. Stevens, Dr. Baker); Memorial Sloan Kettering Inst., NYC, NY (Dr. Gold); LIG, NIAID, NIH, Bethesda, MD (Dr. Folks)

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Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

0.2

PROFESSIONAL

0.1

OTHER

0.1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A new medical syndrome, Acquired Immune Deficiency Syndrome (AIDS) has recently been recognized. It is characterized by profound progressive depression of the immune system, resulting in repeated opportunistic infections and at least one type of neoplasm.

The syndrome is usually if not always fatal. It is epidemic in the United States among certain "high-risk populations (male homosexuals, illicit drug users, Haitians, and, to a lesser extent, hemophiliacs, recipients of blood transfusions and intimate contacts of cases). The epidemiology of the syndrome suggests that it is caused by a transmissible agent. Attempts to transmit a putative AIDS agent to chimpanzees and monkeys are in progress.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00404-01 LID

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Second and Third-Generation Hepatitis B Vaccines

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Robert H. Purcell, M.D. Head, HV Section LID, NIAID

COOPERATING UNITS (if any)

Georgetown University, Washington, DC (Dr. Gerin); Scripps Institute, La Jolla, California (Dr. Lerner); LBV, NIAID, NIH, Bethesda, MD (Dr. B. Moss)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

0.1

PROFESSIONAL:

0.1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Expense, availability and/or incomplete acceptance based upon an unfounded fear of infection with the agents of AIDS limit the impact of plasma-derived hepatitis B vaccines in developed and developing countries. There is therefore a need for new approaches to vaccine development. Recombinant DNA and synthetic peptide technologies appear to offer the best opportunities for the next generation of hepatitis B vaccines. Partial or complete protection against hepatitis B has been demonstrated in chimpanzees following vaccination with (a) recombinant derived subunit vaccine prepared in eukaryotic cells, (b) live recombinant vaccinia virus containing HBV genes, and (c) synthetic peptides representing HBsAg sequences. Attempts to identify the antigenic domains most important in stimulating neutralizing antibody are currently in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 A1 00405-01 LID

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Inactivation of Hepatitis Viruses in Pooled Plasma Derivatives

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Robert H. Purcell, M.D. Head, HV Section LID, NIAID

COOPERATING UNITS (if any)

Georgetown U., Washington DC (Dr. Gerin); Southwest Fndt.,
San Antonio, TX (Dr. Eichberg); Revlon Inc., Tuckahoe, NY (Dr. Landaburu)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

0.2

PROFESSIONAL

0.1

OTHER

0.1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The transmission of viral hepatitis by plasma derivatives continues to be an important medical problem that has been made more urgent by the similar epidemiology of the putative agent of acquired immune deficiency syndrome. Methods of inactivating viruses in plasma derivatives while retaining the biological potency of the products are being explored. These include the application of heat to lyophilized biological products and extraction of lyophilized or aqueous preparations with lipid solvents such as chloroform.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00406-01 LID

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Structure of HAV: Genomic Sequence and Organization

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---------|--------------------------|----------------------|------------|
| PI: | Bahige M. Baroudy, Ph.D. | Visiting Associate | LID, NIAID |
| Others: | Robert H. Purcell, M.D. | Head, HB Section | LID, NIAID |
| | John R. Ticehurst, M.D. | Medical Staff Fellow | LID, NIAID |
| | Steven Feinstone, M.D. | Medical Officer | LID, NIAID |

COOPERATING UNITS (if any)

Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, NIH, Bethesda, Maryland (Dr. Jacob V. Maizel).

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.7

PROFESSIONAL:

1.0

OTHER:

0.7

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have sequenced several HAV cDNA clones by the Maxam and Gilbert technique and to date the sequence corresponding to two large regions of the genome has been determined. A sequence of 3119 bases corresponding to the 5' end of HAV RNA was determined. This sequence contains an open reading frame that begins \approx 750 bases from the 5' terminus and extends 2407 bases which is, as far as sequence has been determined. Sequence preceding this major reading frame has nine other potential initiation sites, but the longest peptide that can be translated in this region is only 26 amino acids. This pattern is consistent with the genomic organization of other picornaviruses. The entire sequence of a clone that mapped to the 3' end of HAV RNA was also determined. A poly(A) tract of 15 bases was found at one end of this clone thus orienting the 3' end of the genome. This poly (A) tract is 51 bases downstream from two closely spaced termination codons that are preceded by 1407 bases in an open reading frame that is presumably continuous with that present at the 5' end of the genome. These sequenced regions of the HAV genome have been compared, using computer programs, to analogous regions previously determined for other picornaviruses.

| | | |
|--|-------------------------------|---------------------------------------|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00322-03 LID |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Amino Acid Sequence of RS Viral Nucleocapsid (NC) Protein | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) | | |
| PI: | Sundararajan Venkatesan, M.D. | Expert LID, NIAID |
| Others: | Narayanasamy Elango, Ph.D. | Visiting Associate LID, NIAID |
| COOPERATING UNITS (if any) | | |
| LAB/BRANCH Laboratory of Infectious Diseases | | |
| SECTION Respiratory Viruses Section | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MAN-YEARS | PROFESSIONAL | OTHER: |
| 0.8 | 0.6 | 0.2 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> We were interested in determining the primary structure of RS viral capsid protein in order to define the different domains of this protein that are involved in RNA-protein interaction. In addition, we wished to understand the genetic organization of this human pathogen at the nucleotide level. Our interest in NC protein stems from a desire to understand the nucleocapsid assembly of RS virus and the various interactions between the NC protein and transcriptional enzymes. Knowledge of the primary structure of the major capsid protein should provide insight into the nature of these interactions. To achieve this goal, we have determined the sequence of this protein. A recombinant plasmid (pRSB₁₁) was selected from a RS cDNA library using screening procedures described elsewhere. DNA sequencing of the RS viral insert by Gilbert-Maxam method revealed an RS virus sequence of 1430 nucleotides excluding the poly(A) tail. By primer extension and dideoxy-sequencing the insert was found to lack only four nucleotides corresponding to the 5' end of the mRNA. Of interest, a nine nucleotide sequence 5'-NGGGCAAAT3' was present at the 5' end of the mRNA strand. This sequence is conserved at the 5' end in all seven RS viral genes sequenced so far. The NC clone has a single long open reading frame of 467 amino acids capable of encoding a protein of 51540 daltons. There was no homology of RS NC sequence with that of VSV, influenza virus, coronavirus and TMV capsid proteins implying that RS virus is evolutionarily distinct. </p> | | |

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00323-03 LID

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure of Parainfluenza Type 3 Virus Genome

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---------|-------------------------------|---------------------|------------|
| PI: | Sundararajan Venkatesan, M.D. | Expert | LID, NIAID |
| Others: | Robert C. Jambou, M.S. | Biologist | LID, NIAID |
| | Narayanasamy Elango, Ph.D. | Visiting Associate | LID, NIAID |
| | Kathleen van Wyke, Ph.D. | Senior Staff Fellow | LID, NIAID |
| | Robert M. Chanock, M.D. | Chief, LID | LID, NIAID |

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.0

PROFESSIONAL:

0.3

OTHER:

0.7

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Poly(A) containing RNA was isolated from cells infected with type 3 parainfluenza virus. Actinomycin D was added to medium to abolish cellular transcription, and RNAs were resolved by agarose gel electrophoresis under denaturing conditions. Nine major poly A(+) RNA's that were estimated to be 0.3, 0.8, 1.4, 1.5, 1.7, 2.0, 2.5, 3.0, and 7.0 kilobases were visualized. Single stranded ³²P labeled cDNAs were synthesized using mRNA's from infected cells and oligo(dT) as primer. When these cDNAs were analyzed by alkali agarose gel electrophoresis, discrete bands ranging in size from about 6000 to 300 bases were observed. The individual single stranded cDNAs were then made double stranded and cloned in *E. coli* by procedures described previously. In addition, RNA/DNA hybrids were cloned directly after dG/dC tailing. Full length cDNA recombinants large enough to represent DNA copies of individual transcripts were identified by serial colony hybridization. Several recombinants encoding different para-3 transcripts have been identified. Northern blot analysis, hybrid-selection and *in vitro* translation are currently being employed to identify the viral recombinants.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00324-03 LID

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Laboratory Studies of Myxoviruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---------|-----------------------------|----------------------|------------|
| PI: | Brian R. Murphy, M.D. | Head, RV Section | LID, NIAID |
| Others: | Robert M. Chanock, M.D. | Chief, LID | LID, NIAID |
| | Mark Snyder, M.D. | Medical Staff Fellow | LID, NIAID |
| | Alicia Buckler-White, Ph.D. | Staff Fellow | LID, NIAID |

COOPERATING UNITS (if any)

LVD, NIAID (Moss, Smith); St. Jude Children's Hospital, Memphis, TN (Hinshaw, Naeve, Webster); Cornell U. School of Vet. Med., Ithaca, NY (Holmes); Scripps Institute, La Jolla, CA (Green, Lerner)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

3.8

PROFESSIONAL

1.8

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Avian-human influenza A reassortant viruses that contain the human influenza hemagglutinin and neuraminidase genes and the six "internal" genes of the A/Mallard/78 (H2N2) avian virus were attenuated for monkeys and man. A similar reassortant derived from the avian A/Pintail/119/79 and the human A/Washington/80 (H3N2) viruses was also attenuated in monkeys and man. The M gene of the A/Mallard/78 virus, which is capable of effecting attenuation by itself, was sequenced and significant divergence from the corresponding gene of a human influenza A virus was observed in the M₂ cistron. The RNA 1 and NS genes of the A/Mallard/78 virus, in combination, also contributed to attenuation of the A/Mallard/78 reassortants viruses for monkeys. Vaccinia-influenza HA recombinants were immunogenic and protective in hamsters.

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| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER <div style="text-align: center;">Z01 AI 00325-03 LID</div> |
| PERIOD COVERED October 1, 1983 through September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Study of Respiratory Viruses in Primates | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) | | |
| PI: | Brian R. Murphy, M.D. | Head, RV Sect. LID, NIAID |
| Others: | Robert M. Chanock, M.D. | Chief, LID LID, NIAID |
| COOPERATING UNITS (if any) Meloy Laboratories, Rockville, MD (Dr. Sly); NINCDS, NIH, Bethesda, MD (Dr. London, Dr. Harper) | | |
| LAB/BRANCH Laboratory of Infectious Diseases | | |
| SECTION Respiratory Viruses Section | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
| 1.4 | 0.4 | 1.0 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Transfer of the six "internal" genes of the avian influenza A/Mallard/78 virus to four human influenza A viruses (one H1N1 strain and three H3N2 strains) reproducibly attenuated the resulting reassortants for monkeys or hamsters. Avian influenza NP and M genes singly or RNA 1 and NS genes in combination play a role in attenuation of these reassortants for monkeys.</p> <p>Transfer of the six "internal" genes of the avian A/Pintail/119/79 influenza virus to the human influenza A/Washington/80 (H3N2) virus also attenuated the resulting reassortant for monkeys. The A/Washington/80 X A/ Pintail/79 reassortant was satisfactorily attenuated, not transmissible, immunogenic, and safe in monkeys. Immunogenicity was demonstrated by the induction of resistance to challenge with wild type virulent human influenza virus. These studies form the basis for evaluation of these promising reassortant viruses in humans.</p> | | |

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|--|-------------------------|---------------------------------------|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00326-03 LID |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Study of Respiratory Viruses in Volunteers | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) | | |
| PI: | Brian R. Murphy, M.D. | Head, RV Sect. LID NIAID |
| Others: | Robert M. Chanock, M.D. | Chief, LID LID, NIAID |
| COOPERATING UNITS (if any) Flow Labs., Rockville, MD; U. of Md. Sch. Med. Balt., MD; U. Rochester Sch. Med., Roch., NY; NCI, Immunology Br., Beth., MD; U. Vt., Brt., VT; Vanderbilt U., Nashville, TN; FDA, Bethesda, MD.; U. Alabama, Birmingham, AL | | |
| LAB/BRANCH Laboratory of Infectious Diseases | | |
| SECTION Respiratory Viruses Section | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MAN-YEARS | PROFESSIONAL: | OTHER: |
| 3.65 | 0.9 | 2.75 |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>An avian human influenza reassortant virus that derived its genes for the surface glycoproteins from the human influenza A/Washington/897/80 (H3N2) virus and its six other genes from the avian influenza A/Mallard/6750/78 virus was safe, satisfactorily attenuated, and non-transmissible in adult volunteers. The human infectious dose₅₀ was 10^{5.5} TCID₅₀. A reassortant virus that derived only its PB1 and M genes from the <u>ca</u> donor was prepared from the cold adapted (<u>ca</u>) A/Ann Arbor/6/60 donor and the A/Washington/80 (H3N2) virus. This reassortant virus was as attenuated as another reassortant that contained all six "internal" genes of the <u>ca</u> donor virus. This suggests that the <u>ca</u> PB1 and M genes contribute to attenuation of <u>ca</u> reassortant viruses for humans. Reassortant <u>ca</u> vaccines containing the six internal <u>ca</u> genes induced resistance to challenge with wild type virus that was demonstrable 6 months later and this resistance exceeded that induced by inactivated influenza virus vaccine.</p> <p>A characterization of the local and serum antibody response to infection or vaccination with inactivated vaccine revealed that: 1) Following primary influenza virus infection heterosubtypic ELISA antibody responses occur. 2) The serum ELISA IgA HA antibody response following infection is mostly polymeric without a secretory piece suggesting a mucosal origin. 3) 95% of volunteers receiving inactivated vaccine parenterally developed a local ELISA IgG HA antibody response.</p> | | |

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| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00327-03 LID |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Characterization of Parainfluenza type 3 Viruses with Monoclones | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) | | |
| PI: | Kathleen L. van Wyke, Ph.D. Senior Staff Fellow | LID, NIAID |
| Others: | Brian R. Murphy, M.D. Head, RV Sect. | LID/NIAID |
| COOPERATING UNITS (if any) Baylor College of Medicine, Houston, TX | | |
| LAB/BRANCH Laboratory of Infectious Diseases | | |
| SECTION Respiratory Viruses Section | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
| 2.2 | 1.2 | 1.0 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Neutralizing monoclonal antibodies to the 1957 prototype parainfluenza type 3 virus have been used to map the epitopes on the hemagglutininneuraminidases (HN) surface glycoprotein and to examine the epidemiology of parainfluenza viruses. Competitive-binding assays indicated that these neutralizing antibodies bind to one antigenic site. Analysis of variants selected in the presence of these antibodies showed that there are 5 distinct epitopes within this area. Examination of parainfluenza type 3 viruses isolated from three geographic regions during the past 25 years showed that antigenic variation occurs but this variation is probably a reflection of genetic heterogeneity among these viruses rather than progressive evolution. Furthermore, all of the variation detected in clinical isolates occurs in only 2 of the 5 detectable epitopes on the HN molecule. | | |

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| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER <div style="text-align: center; font-weight: bold;">Z01 A1 00344-03 LID</div> |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Immunologic Characterization of Respiratory Syncytial Viruses | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) | | |
| PI: | Gregory A. Prince, D.D.S., Ph.D. Expert | LID, NIAID |
| Others: | Robert M. Chanock, M.D. Chief, LID | LID, NIAID |
| COOPERATING UNITS (if any) | | |
| LAB/BRANCH Laboratory of Infectious Diseases | | |
| SECTION Respiratory Viruses Section | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
| 0.85 | 0.35 | 0.5 |
| CHECK APPROPRIATE BOX(ES) | | |
| <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) | | |
| <p>Cotton rats previously inoculated with formalin-inactivated RSV were challenged intranasally with live RSV in an attempt to experimentally induce a potentiated RSV disease similar to that observed following administration of formalin-inactivated RSV vaccine to infants 20 years ago. Within 24 hours after infection with RSV cotton rats developed pulmonary lesions that reached a maximum by the fourth day. Histologically the lesions resembled an experimental pulmonary Arthus reaction, although adoptive transfer experiments were not successful in defining their pathogenesis. A similar immunopathologic reaction also developed in cotton rats which were challenged with live type 3 parainfluenza virus following vaccination with formalin-inactivated parainfluenza-3 virus. The action of formalin on RSV and parainfluenza 3 virus appears to be responsible for triggering the immunopathologic reactions, because heat inactivation in the absence of formalin, did not confer on virus the ability to induce immunopathology.</p> | | |

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00345-03 LID

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunity to RS Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Gregory Prince, D.D.S., Ph.D. Expert LID, NIAID

Others: Robert M. Chanock, M.D. Chief LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

0.85

PROFESSIONAL:

0.35

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The cotton rat was used as an experimental animal to test the protective efficacy of passively acquired serum neutralizing antibodies to RS virus (RSV). Infant cotton rats inoculated with varying amounts of homologous convalescent RSV antiserum were subsequently challenged intranasally with RSV. Cotton rats with a circulating titer of neutralizing antibody of 1:350 or greater at the time of challenge were completely resistant to pulmonary infection; cotton rats with a titer of 1:80 to 1:350 became infected but the level of virus replication in the lung was reduced in direct relation to the titer of antibody; while animals with an antibody titer less than 1:80 sustained an undiminished RSV infection in the lungs. The nose was not protected against RSV even by the highest level of passive serum antibodies. These observations suggest that maternally-derived antibodies can exert a protective effect against RSV infection in the lungs, however, a very high concentration of antibodies is required for significant protection in the lower respiratory tract. The concentration of passive serum antibodies in the circulation of young human infants decreases approximately 50% every month. This may serve to explain the relative sparing of very young infants from serious RSV disease. Within a few months after birth the concentration of passively acquired RSV antibodies drops below the critical threshold, 1:80-1:350, and the lungs lose their resistance.

Suspensions of purified human IgG (Sandoglobulin) were shown to contain a very high level of antibody to RSV. Sandoglobulin administered intraperitoneally to cotton rats prevented pulmonary infection with RSV. Possibly more significant was the therapeutic effect of human RSV antibodies administered after infection had become well established. Administration of lesser amounts of Sandoglobulin to RSV-infected owl monkeys also decreased the level of virus in the lungs over the next 2 days. These observations suggest that serious RSV disease in infants may respond to immunotherapy.

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| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00367-02 L10 |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) RS Viral Nonstructural Proteins | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) | | |
| PI: | Sundararjan Venkatesan, M.D. | Expert LID, NIAID |
| Others: | N. Elango, Ph.D. M. Satake, Ph.D., M.D. | Visiting Associate LID, NIAID Visiting Associate LID, NIAID |
| COOPERATING UNITS (if any) | | |
| LAB/BRANCH Laboratory of Infectious Diseases | | |
| SECTION Respiratory Viruses Section | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
| 0.6 | 0.5 | 0.1 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Recent studies indicate that two RS viral nonstructural proteins (NS1 and NS2) are coded for by two adjacent genes. By means of positive hybrid selection of viral mRNAs and subsequent <i>in vitro</i> translation of the selected RNAs a recombinant RS viral plasmid (pRSC₆) that encoded two viral nonstructural proteins was identified. This plasmid that contained a RS viral insert of about 1,050 bases hybridized to viral mRNAs containing about 500-600 bases as indicated by Northern blot analysis. The insert was sequenced completely and found to contain two different nonoverlapping reading frames that coded for proteins that contained 139 and 124 amino acids respectively. Upstream of the second reading frame there was a nine nucleotide GGGGCAAAT sequence between positions 545-553. The recombinant pRSC₆ was thus shown to be a cDNA clone of a bicistronic transcript. Bicistronic transcript(s) of RS virus may originate because of a failure of the viral polymerase to pause at the intergenic region.</p> | | |

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00368-02 LID

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structural Analysis of Respiratory Syncytial Virus Genome

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Sundararajan Venkatesan, M.D. Expert LID, NIAID

Others: Narayanasamy Elango, Ph.D. Visiting Associate LID, NIAID
Masanobu Satake, M.D., Ph.D. Visiting Associate LID, NIAID
Robert M. Chanock, M.D. Chief, LID LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

0.8

PROFESSIONAL:

0.6

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Human respiratory syncytial (RS) virus, an enveloped non-segmented negative strand RNA virus, is the major etiologic agent of severe lower respiratory illness of infants and young children. We have used recombinant DNA techniques and biochemical analysis to explore the genome of this virus. A cDNA library representing the poly(A) containing RNA's from RS virus infected cells was constructed in *E. coli* (HB101) using plasmid pBR322 as a vector. The strategy for cloning avoided the traditional approach that includes a self priming reaction for second strand synthesis and subsequent *S₁* nuclease treatment to remove the resulting hairpin. We have determined the complete DNA sequences of six RS viral genes cloned in recombinant plasmids. A seventh gene that appears to code for the fusion protein, has also been sequenced; this recombinant lacks about 70 nucleotides corresponding to the 5' end of the message. There was conservation of a PuGGGCAAAAT....sequence at the 5' end of the 7 RS viral transcripts. This consensus initiation sequence differs from that found at the 5' end of paramyxovirus and rhabdovirus transcripts. The initiating nucleotide is probably G in all RS virus genes.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00371-02 LID

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Nucleotide Sequence of the Genes Encoding RS Viral M and P Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---------|-------------------------------|--------------------|------------|
| PI: | Sundararajan Venkatesan, M.D. | Expert | LID, NIAID |
| Others: | Masanobu Satake, M.D., Ph.D. | Visiting Associate | LID, NIAID |
| | N. Elango, Ph.D. | Visiting Associate | LID, NIAID |

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

0.7

PROFESSIONAL:

0.6

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
 ☐ (b) Human tissues
 ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We determined the amino acid sequence of RS viral M protein was deduced from the cDNA sequence of a recombinant plasmid harboring the gene. The RS viral cDNA insert of 950 nucleotides had a poly(A) tail at one end. The other end corresponding to the 5' end of the mRNA lacked five nucleotides (NGGGC) of the mRNA. The cDNA sequence had an open reading frame capable of encoding a protein of 28717 dal (256 amino acids). The protein was relatively basic and moderately hydrophobic. It did not contain regions homologous to other viral matrix proteins. A second open reading frame potentially encoding a protein of 75 amino acids was also present at the 3' end of the cDNA insert. This overlapped the first reading frame by 20 amino acids. Several recombinant plasmids containing cDNA encoding the RS viral phosphoprotein gene were identified by a variety of methods. pRSA₃ encoding RS viral P protein was selected for sequencing. It has 916 bp of RS viral sequence including a poly A tail of 14 residues. It lacked the NGGG...sequence corresponding to the 5' end of the mRNA. As with other RS viral genes this is part of the conserved sequence 5' NGGGCAAAT. Starting at position 18, there is a single long open reading frame encoding a protein of 241 amino acids with a molecular weight of 27150. It lacks sequence homology with Sendai virus P protein or VSV NS protein which represent counterparts of RS P protein. Unlike the situation reported for Sendai and measles viruses, this gene does not have a second reading frame capable of encoding another protein.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00372-02 LID

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Respiratory Syncytial Virus Glycoproteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Sundararajan Venkatesan, M.D. Expert LID, NIAID

Others: Narayanasamy Elango, Ph.D. Visiting Associate LID, NIAID
Masanobu Satake, M.D., Ph.D. Visiting Associate LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

0.7

PROFESSIONAL:

0.5

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

RS virus possesses two glycoproteins; a 84-88 kdal glycoprotein that lacks hemagglutinin and neuraminidase activities and is referred to as G. The other is a 68 kdal protein that is thought to be the fusion factor(F_0); F_0 is proteolytically cleaved into two subunits, F_1 (48 kdal) and F_2 (16 kdal). Since an amino acid sequence for these proteins is not available, we have attempted to deduce this sequence by a combination of recombinant DNA technology and DNA sequencing. A RS viral cDNA insert of 1755 bp hybridized to a viral poly(A) RNA species 2000-2200 bases long. An RNA of this size was shown by Collins and Wertz to be translated in vitro to a 59 kdal protein, the putative unglycosylated translation progenitor of the 68 kdal viral fusion glycoprotein. The cDNA insert lacked a poly(A) tail. However, the messenger strand in the insert was established by primer extension of an asymmetrically 5' end labeled restriction fragment on mRNA from infected cells. Dideoxy-sequencing with this primer indicated that the recombinant lacked approximately 70 nucleotides corresponding to the 5' end of the mRNA. pRSA₁₄ has a single long open reading frame, starting at position 1 and ending at position 1671, that is capable of encoding a partial protein of 556 amino acids. Inspection of the amino acid sequence near the C-terminus revealed a 24 amino acid sequence extremely rich in hydrophobic residues followed by a 25 amino acid sequence rich in hydrophilic and polar residues extending all the way to the C-terminus of the protein.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00328-02 LID

PERIOD COVERED

October 1, 1982 through September 30, 1983

TERMINATED

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Defects of Influenza Hemagglutinins Altered at the Hydrophobic Carboxy-Terminus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Michael M. Sveda, Ph.D.

Expert

LID NIAID

Others: L. Markoff, M.D.
C.-J. Lai, Ph.D.Medical Officer
Head, MVB SectionLID NIAID
LID NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

1.0

PROFESSIONAL:

0.7

OTHER

0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Requirements for cell surface expression of the influenza hemagglutinin (HA) were studied using a recombinant of SV40 which had full-length DNA sequences coding for the influenza virion HA inserted into the late region of SV40 DNA and propagated in the presence of tsA SV40 helper. Infection of primate cells with the SV40-HA recombinant produced a functional glycosylated HA polypeptide that accumulated on the cell surface. To delineate the protein domains necessary for surface expression of the HA polypeptide, mutations were engineered in the recombinant SV40-HA DNA. One mutant of interest sustained a deletion of 5 base pairs at the Bam HI site generating a shift in reading frame for the hydrophobic sequence of 24 amino acids as well as the C' terminal 13 amino acids. This shift yielded a terminal sequence in which 21 of 24 amino acids were hydrophobic or neutral followed by 4 charged amino acids. Although its C' terminus resembled wild-type topologically, the mutant HA was not secreted, was not expressed on the cell surface, and was only present intracellularly. These findings suggest a role for the hydrophobic COOH-terminus not only in cell surface expression but in secretion related glycosylation.

T E R M I N A T E D

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00329-03 LID

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Expression of the Influenza A Virus Neuraminidase Glycoprotein from Cloned DNA

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---------|----------------------|-------------------|------------|
| PI: | Lewis Markoff, M.D. | Medical Officer | LID, NIAID |
| Others: | Erich Mackow, Ph.D. | Staff Fellow | LID, NIAID |
| | Ching-Juh Lai, Ph.D. | Head, MVB Section | LID, NIAID |

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

National Institutes of Health, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

0.7

PROFESSIONAL:

0.5

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A full-length ds DNA copy of the virion RNA segment coding for an influenza A neuraminidase (NA) glycoprotein was previously cloned into the late (deleted) region of an SV40 shuttle vector. The influenza-specific product of a lytic infection with this vector was shown to be glycosylated and inserted in the outer cell membrane. Additional studies established that weak enzymatic activity of the vector-coded NA was detectable in lysates of infected cells. Three deletion mutant NA DNAs that lacked sequences coding for 7 (dIK), 21 (dII) or all 23 amino acids (dIZ) of the N-terminal hydrophobic region in the wild-type NA were studied in similar fashion, and a comparison of the phenotypes of these mutants suggested that this region functions not only in membrane anchorage but also as a signal sequence, permitting entry of the nascent NA polypeptide into membrane organelles for glycosylation. Experiments are now in progress to induce point mutations in DNA coding for the hydrophobic N-terminus of the NA protein to determine: (1) whether the membrane anchorage function and the "signal sequence" function of this sequence can be altered separately and (2) whether the strict conservation of the N-terminal 12 residues in this region among all influenza A strains has special significance.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00330-02 LID

PERIOD COVERED

October 1, 1982 through September 30, 1983

TERMINATED

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Expression of Influenza Virus Nucleoprotein Using an SV40 Vector

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Bor-Chian Lin, Ph.D.

Visiting Associate

LID NIAID

Others: Ching-Juh Lai, Ph.D.

Head, MVB Section

LID NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

0.7

PROFESSIONAL:

0.5

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

An influenza A virus nucleoprotein (NP) gene was cloned from DNA sequences derived by reverse-transcription of virion RNA segments using synthetic oligonucleotide primers as detailed previously. The cloned NP DNA coding for a full-length copy of a virion NP RNA segment was verified by the size and the completeness of DNA sequences at both terminal ends. The influenza A virus nucleoprotein (NP) gene was cloned into the BamHI site of the late region of SV40 in an SV40-pBR322 expression vector. African green monkey kidney primary cells transfected with the SV40-NP recombinant DNA in the presence of an early SV40 *ts* mutant helper, synthesized a polypeptide that was specifically immunoprecipitable with NP monoclonal antibodies and that had a molecular weight of 56 K daltons identical to the NP of influenza virus as estimated on SDS-polyacrylamide gels. The putative NP was detected in the nucleus of infected primate cells by an indirect immunofluorescence assay. This nuclear localization of NP from recombinant DNA was similar to that seen during influenza virus infection suggesting the NP product may be functionally active.

T E R M I N A T E D

| | | |
|--|---|--|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00331-03 LID |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Transcription of Influenza A Virus: Synthesis of Spliced and Unspliced mRNAs | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) | | |
| PI: Ching-Juh Lai, Ph.D. | Head, MVB Section | LID, NIAID |
| Others: Robert M. Chanock, M.D. | Chief, LID | LID, NIAID |
| COOPERATING UNITS (if any) Robert A. Lamb, Ph.D., Dept. of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, Illinois | | |
| LAB/BRANCH Laboratory of Infectious Diseases | | |
| SECTION Molecular Viral Biology Section | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MAN-YEARS: <div style="text-align: center;">0.1</div> | PROFESSIONAL: <div style="text-align: center;">0.1</div> | OTHER: <div style="text-align: center;">0.0</div> |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Influenza virus RNA segment 8 codes for two distinct proteins, NS₁ and NS₂, that are translated from separate mRNA's. Mapping and sequencing studies have shown that the NS₁ mRNA is a colinear transcript and NS₂ mRNA contains a spliced region. In order to investigate the splicing potential of influenza virus mRNA derived from virion RNA segment 8, cloned full-length NS DNA was inserted into the late region of an SV40 expression vector and the recombinant used for infection of primate cells. Sizing by nuclease S₁ analysis and nucleotide sequencing indicated that both interrupted and uninterrupted mRNA's containing influenza NS sequences were synthesized in cells infected with the recombinant. The sequences found at the junction of the interrupted mRNA were identical to those found in the NS₂ mRNA produced in influenza virus infected cells. These studies establish that during influenza virus infection processing of the NS mRNA transcript involves a mechanism of splicing similar to that which occurs with DNA-directed RNA transcription. Our observations thus eliminate other possible explanations for interrupted mRNAs such as "transcription from defective interfering particles and transcriptional jumping." </p> | | |

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00332-03 LID

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Engineering the Genome of Influenza Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---------|-------------------------|-------------------|------------|
| PI: | Ching-Juh Lai, Ph.D. | Head, MVB Section | LID, NIAID |
| Others: | Lewis J. Markoff, M.D. | Medical Officer | LID, NIAID |
| | Kevin Ryan, Ph.D. | Staff Fellow | LID, NIAID |
| | Erich Mackow, Ph.D. | Staff Fellow | LID, NIAID |
| | Robert M. Chanock, M.D. | Chief | LID, NIAID |

COOPERATING UNITS (if any)

The Wistar Inst., Philadelphia, Pa. (Jonathan Yewdell)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

0.8

PROFESSIONAL:

0.7

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

Our goal has been to use recombinant DNA techniques to construct influenza virus mutants with deletions in strategic regions of the genome. Viable deletion mutants would be especially valuable for use in immunoprophylaxis since these mutants would be unlikely to revert and therefore should be stable as regards phenotype. With this goal in mind, we produced full-length cloned DNA sequences of gene segments of an H3N2 influenza A virus. Thus far we have cloned and characterized 6 full-length genes (hemagglutinin (HA), neuraminidase (NA), nucleoprotein (NP), matrix protein (M), non-structural proteins (NS), and polymerase protein PB2); the remaining two genes (PB1 and PA) have also been cloned but not in complete form. These full-length DNA clones should produce corresponding RNA transcripts that contain the control sequences needed for transcription and replication of viral genes. The validity of this prediction was established for transcription and expression of viral proteins. Functional influenza viral protein (HA, NA, or NP) was produced when simian cells were transfected with a SV40 recombinant vector containing cloned influenza DNA. The influenza cDNA was inserted into the late region of SV40 in an orientation which resulted in transcription of (+) strand influenza RNA. Attempts to rescue cloned influenza DNA by coinfection of transfected cells with influenza A virus were unsuccessful.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00365-02 LID

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Functional Analysis of Influenza Hemagglutinin

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Ching-Juh Lai, Ph.D. Head, MVB Section LID, NIAID

Others: Kenji Sekikawa Visiting Associate LID, NIAID
Joanna Hansen Staff Fellow LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

0.4

PROFESSIONAL:

0.2

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We employed site-specific mutagenesis to generate a series of mutants of an HA-SV40 recombinant. These mutants contained point mutations in the region of the HA gene that encodes the signal peptide sequences. The mutant HA-SV40 recombinants were then used to transfect African green monkey kidney cells in order to achieve expression of mutant hemagglutinins. Characterization of mutant influenza virus HAs with altered signal peptide revealed that a majority of the mutations had no effect on functional properties of HA such as cell surface expression and erythrocyte binding. However, one mutant (designated mutant 28) that sustained multiple amino-acid substitutions produced HA that remained intracellular and was not expressed on the cell surface. Sequence comparison of this mutant and other mutants that were expressed normally on the cell surface suggested that amino acid substitution at the signal cleavage site was responsible for the observed functional abnormality. The defective intracellular HA contained an endoglycosidase H (endo H) sensitive carbohydrate component, whereas the endo H resistant sugar moiety normally present in the wild type HA was not detected. In addition, the molecular size of non-glycosylated mutant 28 HA was larger than non-glycosylated wild type HA as indicated by a slower migration rate on SDS-polyacrylamide gel. This finding is consistent with the sequence data indicating that the mutant HA was altered at the signal cleavage site. These results suggest that HA containing uncleaved hydrophobic signal sequences translocates across the microsomal membranes but fails to undergo further transport to the golgi apparatus where additional processing of carbohydrate components takes place.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00366-02 LID

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Molecular Biology of Dengue Viruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---------|----------------------|--------------------|------------|
| Co-PI: | Ching-Juh Lai, Ph.D. | Head, MVB Section | LID, NIAID |
| | Banghti Zhao, Ph.D. | Visiting Associate | LID, NIAID |
| Others: | Robert M. Chanock | Chief, LID | LID, NIAID |

COOPERATING UNITS (if any)

Dept. Virus Diseases, Walter Reed Army Inst. of Research,
Washington, D.C. (Dr. Walter Brandt)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

1.2

PROFESSIONAL

1.1

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Dengue viruses are members of the flavivirus group of togaviridae that contain a positive stranded RNA genome of approximately 10-12 kilobases. We employed recombinant DNA techniques to investigate the molecular biology of dengue virus with the intent of developing immunoprophylactic measures against this virus group that is epidemic in many geographical areas. The 42S full-length RNA from dengue virus type 4, produced in C6/36 mosquito cells, was isolated and tailed with poly(A) at the 3'-terminus using *E. coli* poly(A) polymerase. Complementary DNA was synthesized by reverse-transcription using oligo(dT) as a primer and subsequently converted to double stranded DNA by oligo d(C) tailing and oligo d(G) priming. The dengue DNA product was inserted into the Pst I site of pBR322 using the dG/dC linker sequences. *E. coli* transformants containing dengue virus specific sequences were identified by in situ colony hybridization. Characterization of recombinant plasmids from several transformants showed that these DNA inserts were short in length ranging from 200 to 300 base pairs. Nevertheless, the dengue specific sequences should prove useful for reverse-transcription extension of virion RNA and replicative form RNA in order to clone longer, and ultimately full-length cDNA copies of the dengue genomic RNA sequence. Cloned dengue virus DNA will be used for: (1) mapping and sequencing of viral genes; (2) synthesis of viral polypeptides; and (3) construction of full-length infectious dengue virus DNA.

| | | |
|--|---|---|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00369-02 LID |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Persistent Expression of Influenza Virus Polymerase Proteins from Cloned DNA | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) | | |
| Co-PI: | Ching-Juh Lai, Ph.D. Erich Mackow, Ph.D. | Head, MVB Section Staff Fellow LID, NIAID LID, NIAID |
| Others: | Kevin Ryan, Ph.D. Lewis Markoff, M.D. | Staff Fellow Medical Officer LID, NIAID LID, NIAID |
| COOPERATING UNITS (if any) | | |
| LAB/BRANCH Laboratory of Infectious Diseases | | |
| SECTION Molecular Viral Biology Section | | |
| INSTITUTE AND LOCATION NIH, Bethesda, Maryland 20205 | | |
| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
| 0.6 | 0.4 | 0.2 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> We sought to identify and characterize naturally occurring deletion mutants in internal genes of influenza virus so that they could be evaluated for their ability to confer attenuation. For example, persistent expression of influenza virus polymerases in permissive cells transfected with cloned influenza DNA should provide a system for complementation and selection of viruses containing mutations in the polymerase genes. The recently developed shuttle vector system that contains a mutant DHFR gene as a selectable marker was employed. Recombinant DNA containing separate promoters for transcription of the influenza PB2 DNA and DHFR DNA was constructed and used for transfection of simian CV-1 cells. The transfected cells were exposed to methotrexate for selection of transfected cells. Methotrexate-resistant cells will be evaluated for stable expression of the PB2 polypeptide by biochemical analysis. </p> | | |

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00407-01 LID

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Persistent Expression of Influenza Virus Cloned DNA

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---------|----------------------|-------------------|------------|
| Co-PI: | Ching-Juh Lai, Ph.D. | Head, MBV Section | LID, NIAID |
| | Kevin Ryan, Ph.D. | Staff Fellow | LID, NIAID |
| Others: | Erich Mackow, Ph.D. | Staff Fellow | LID, NIAID |

COOPERATING UNITS (if any)

Wistar Institute, Philadelphia, Pa. (Dr. Jonathan Yewdell)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

0.8

PROFESSIONAL

0.7

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A cloning strategy was devised for construction of recombinants of cloned influenza NP DNA (coding for the nucleoprotein) and a bovine papilloma virus DNA vector. This NP-BPV recombinant DNA was used for transfection of a mouse cell line in order to establish persistent expression of influenza nucleoprotein. Recombinant DNA transfected cells were morphologically transformed and produced NP that was localized mainly to the nucleus during early passage of transfected cell cultures. Subcloning of transformants yielded a heterogeneous cell population and NP exhibited a more cytoplasmic distribution. These observations suggest that persistent expression of NP was accompanied by alteration of cell phenotype. When analyzed by sucrose gradient sedimentation the NP product was detected in the low molecular weight fractions suggesting that little or no encapsidation of RNA by NP had occurred. Full-length NP was immunoprecipitated with specific antiserum from cell lysates of transformants. This NP was phosphorylated similar to NP produced during influenza virus infection. Other techniques for establishing persistent expression of influenza NP DNA and other influenza cloned DNAs are under study. These include a simian cell system which is permissive for influenza virus infection so that complementation and gene rescue can be attempted. In these studies a vector that contains dihydrofolate reductase (DHFR) DNA as a selectable marker is being used for transfection and persistent expression.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00408-01 LID

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Attempts at Allele Replacement of NA Gene

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Lewis J. Markoff, M.D. Medical Officer

LID, NIAID

Others: Ching-Juh Lai, Ph.D. Head, MVB Sect.

LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

0.7

PROFESSIONAL:

0.5

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A major goal of our laboratory has been and continues to be the application of recombinant DNA technology to the development of an effective live influenza virus vaccine. A suitable donor strain must meet defined criteria of reduced virulence, lack of transmissibility, and stability. Such a master strain would be used as a source of attenuating genes for newly emerged virulent antigenic variants.

We attempted to rescue the NA gene DNA cloned in both the (+) and (-) sense orientation in the late region of our pSV2330 shuttle vector. This vector contains SV40 late 19S and 16S mRNA splice sequences and transcription termination sites. We succeeded, however, in only isolating antigenic variants of the N1 NA that had escaped neutralization. Data from Dr. Webster's laboratory showed that such variants within a single epitope occur with a frequency of 10^{-5} in vitro. The "rescue" event in this experiment entails the statistically much less likely acquisition by replicating WSN virus of N2 NA virion RNA derived from cloned influenza DNA.

The conservation of a 12-base sequence at the 3'-end and a different 13-base sequence at the 5'-end of all influenza A vRNAs suggests that the termini are crucial to certain aspects of viral replication, perhaps recognition by viral polymerase(s) for the production of full-length (-) strands or perhaps recognition for packaging by nucleoprotein. In the foregoing attempts at rescue, the influenza sequences were transcribed as part of a "fusion" mRNA that included SV40 sequences both upstream and downstream from the NA sequence. Thus the common termini in influenza vRNA were not available as free ends to the viral replication complex.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00409-01 LID

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less: Title must fit on one line between the borders.)

Cloning and Expression of Influenza Virus Polymerase Genes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---------|----------------------|-------------------|------------|
| Co-PI: | Ching-Juh Lai, Ph.D. | Head, MVB Section | LID, NIAID |
| | Erich Mackow, Ph.D. | Staff Fellow | LID, NIAID |
| Others: | Lewis Markoff, M.D. | Medical Officer | LID, NIAID |

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

0.8

PROFESSIONAL

0.4

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

We have cloned a full-length copy of double stranded DNA that codes for an influenza virus polymerase protein, PB2. PB2 is one of the two basic proteins that are present in small copy number in the viral nucleocapsid. PB2 binds and then cleaves capped host cell mRNA. This represents the first step in the priming of influenza viral mRNA transcription. We have initiated attempts express functional PB2 in mammalian cells. Initially, cloned PB2 DNA flanked by Bam HI linker sequences was inserted into the late region between the Hpa II and the Bam HI sites of an SV40 vector that contains a viable deletion in the small t region; this deletion provides additional room for packaging foreign DNA in SV40. Recombinant PB2-SV40 DNA was used for transfection of primate cells and was successfully propagated in the presence of an early SV40 ts mutant helper. Synthesis of the PB2 polypeptide in recombinant infected cells is currently being analyzed by immunoprecipitation and by in vitro translation of PB2 specific mRNA. A recombinant that expresses polymerase PB2 should be useful for determining whether PB2 by itself exhibits the functional activity that has been ascribed to it as a component of the nucleocapsid transcriptase complex. In addition, complementation analysis of ts influenza mutants will be carried out to test the biologic activity of PB2 produced in recombinant infected cells.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00333-03 LID

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

A Longitudinal Study of Viral Gastroenteritis in Infants and Young Children

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Albert Z. Kapikian, M.D. Head, Epid. Sect. LID NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

0.4

PROFESSIONAL:

0.1

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Rotaviruses have been studied extensively by predominantly cross-sectional approaches. Such studies have yielded essentially "numerator" data which indicated that rotaviruses are a major cause of diarrheal illness. There have been few longitudinal gastroenteritis studies yielding important epidemiologic information. Therefore we initiated an examination of anal swab and serum specimens obtained during a previous long-term longitudinal study (1955-1969) at Junior Village, a welfare institution for normal, homeless children. Anal swabs and blood specimens were obtained routinely. Surveillance was carried out by a trained medical staff. As reported previously, 139 rotavirus strains have been detected with the characteristic seasonal distribution. It should be possible to establish the serotypic diversity of these strains. The subgrouping pattern was consistent with other studies as most of the tested strains belong to subgroup 2. In addition, as noted previously, sequential sera from 384 children in residence sometime between May 19, 1963-May 31, 1966 have been tested for CF antibody to the "0" agent. 150 (40%) of the children experienced at least one rotavirus infection; 11 had a second infection and one a third infection. For the period from May 22, 1966-May 21, 1969 65 (36%) of 182 children (some overlap with previous period) experienced at least one rotavirus infection, with 6 having a second infection. We will attempt to propagate selected rotavirus positive specimens in tissue culture by direct isolation or genetic reassortment in order to serotype them.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00334-03 LID

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Monoclonal Antibodies to Proteins of Rotavirus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Karen Midthun, M.D. Medical Staff Fellow LID, NIAID

Others: Jon Askaa, D.V.M. Guest Worker LID, NIAID
Albert Kapikian, M.D. Head, Epid. Section LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

0.7

PROFESSIONAL:

0.2

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In an attempt to clarify the nature of rotavirus antigens and as a means of investigating rotavirus protein structure and function, we have isolated a series of monoclonal antibodies directed at several rotavirus proteins. Monoclonal antibodies with varying specificities were derived from mice immunized with the Wa strain of human rotavirus (serotype 1), rhesus rotavirus (RRV) #2, or a reassortant rotavirus (ts bovine UK x DS-1) which has the human serotype 2 neutralization specificity. Monoclonal antibodies were produced using standard hybridoma technology. Balb/C mice were immunized 3-4 times over the course of approximately 3 months with partially purified virus. The fusion ratio was 10 spleen cells per NS-1 myeloma cell. A solid phase radioimmunoassay (RIA) using either homologous or heterologous virus was the primary screening assay. Screening by hemagglutination-inhibition (HI) assay was a key adjunct to identifying monoclonal antibodies directed at the major outer capsid protein of RRV. Positive samples were cloned once or twice by terminal dilution, grown in culture for study and inoculated into mice for production of ascites fluid. Monoclonal antibodies were further characterized in immunoprecipitation experiments with viral lysates, in plaque reduction neutralization assays (PRNA), in solid phase RIA and HI assays using reassortant rotaviruses with predetermined genotype as antigens, and by immune electron microscopy (IEM).

| | | |
|---|--------------------------|---------------------------------------|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00335-03 LID |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Rotavirus Reassortants: Genetics and Use in Rotavirus Vaccines | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) | | |
| PI: | Karen Midthun, M.D. | Medical Staff Fellow LID, NIAID |
| Others: | Yasutaka Hoshino, M.D. | Visiting Associate LID, NIAID |
| | Richard Wyatt, M.D. | Medical Officer LID, NIAID |
| | Jorge Flores, M.D. | Expert LID, NIAID |
| | Albert Z. Kapikian, M.D. | Head, Epid. Sect. LID, NIAID |
| | Robert Chanock, M.D. | Chief, LID LID, NIAID |
| COOPERATING UNITS (if any) | | |
| LAB/BRANCH Laboratory of Infectious Diseases | | |
| SECTION Epidemiology Section | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
| 1.8 | 0.9 | 0.9 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Rotaviruses, newly classified members of the Reoviridae family, are an important cause of infantile diarrhea in a wide variety of mammalian species including man. Because they have a segmented genome, rotaviruses undergo genetic reassortment at high frequency during mixed infection. Rotavirus reassortants have been used to establish gene coding assignments for several major antigenic specificities and functional activities of these viruses. In addition, reassortants with characteristics that make them potential vaccine candidates have been isolated. We succeeded in isolating a large number of reassortant viruses following coinfection of cell cultures with a fastidious human rotavirus - D, DS-1, P, or ST3 (serotype 1, 2, 3, or 4) and a wild type bovine or rhesus rotavirus. These reassortants were specifically isolated as vaccine candidate strains. Wild type animal rotaviruses were used since these viruses are less likely to have silent point mutations than mutagenized ts virus. Analysis of the genotype of these reassortants indicates that many reassortants possess 10 genes of animal rotavirus origin and a single gene of human rotavirus origin. These single human rotavirus gene substitution reassortants have the serotype specificity of their human rotavirus parent as determined by plaque reduction neutralization assay. Such reassortants with a single human rotavirus gene substitution represent promising candidate live vaccine strains. Because of the narrow and highly restricted host range observed for rotaviruses recovered from a wide variety of species, it is likely that substitution of a large number of animal rotavirus genes for the corresponding genes of human rotavirus will lead to attenuation for humans. On the other hand the major protective antigen would be derived from the human rotavirus parent. These reassortants have been adapted to growth in DBS FRHL-2 cells. Successful passage in such a diploid cell strain further enhances their potential as vaccine candidate strains. </p> | | |

| | | |
|---|---|--|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00338-03 LID |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular Cloning of Rotavirus Genes | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) | | |
| PI: | Jorge Flores, M.D. | Expert LID, NIAID |
| Others: | Jerry Keith, Ph.D. Roger Glass, M.D. Osamu Nakagomi, M.D., Ph.D. Toyoko Nakagomi, M.D. Ph.D. Albert Z. Kapikian, M.D. Robert M. Chanock, M.D., | Staff Fellow LID, NIAID Medical Officer LID, NIAID Visiting Fellow LID, NIAID Visiting Fellow LID, NIAID Head, Epid. Sect. LID, NIAID Chief, LID LID, NIAID |
| COOPERATING UNITS (if any) | | |
| LAB/BRANCH Laboratory of Infectious Diseases | | |
| SECTION Epidemiology Section | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MAN-YEARS | PROFESSIONAL | OTHER |
| 4.2 | 3.3 | 0.9 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) | | |
| <p> We made cDNA copies of genes of three different rotavirus strains (the human Wa [serotypic 1], the bovine NCDV and the simian rhesus Rh2 [serotype 3]) were prepared by reverse transcribing their genomic RNAs or single stranded RNAs synthesized <u>in vitro</u> from rotavirus particles. The cDNAs were tailed and inserted into pBR322 and the resulting recombinants were used to transform <u>E. coli</u>. The genes from which most of these clones were derived were identified by means of a dot hybridization assay. Clones containing copies of each rotavirus gene (except for genes 10 and 11) have been identified. The size of the rotavirus cDNA inserts in the plasmids were determined for more than half of all the clones obtained (more than 2500). Rotavirus cDNAs that may represent full size copies of genes 5-9 have been identified and their restriction patterns analyzed. Some of them are currently being sequenced in order to deduce the amino acid sequences of the proteins they encode and to facilitate their insertion into expression vectors. </p> <p> Synthesis of the relevant protective antigens in bacteria or yeast directed by expression vectors containing cloned DNA copies of the appropriate rotavirus genes may provide abundant amounts of antigen for use in a vaccine. Expression of such antigens as fusion proteins on the surface of bacteria which could transiently colonize the small intestine may stimulate local immunity and provide protection. Alternatively, synthetic peptides representing the major antigenic determinants responsible for the induction of neutralizing antibody could be used for immunoprophylaxis. </p> | | |

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|--|---|---|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER <div style="text-align: center;">201 AI 00339-03 LID</div> |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Isolation in Cell Culture and Serotypic Characterization of Human Rotaviruses | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) | | |
| PI: | Yasutaka Hoshino, D.V.M. Richard G. Wyatt, M.D. | Visiting Associate Medical Officer LID, NIAID LID, NIAID |
| Others: | Jorge Flores, M.D. Albert Z. Kapikian, M.D. Robert M. Chanock, M.D. | Expert Head, Epid. Sect. Chief, LID LID, NIAID LID, NIAID LID, NIAID |
| COOPERATING UNITS (if any) | | |
| LAB/BRANCH Laboratory of Infectious Diseases | | |
| SECTION Epidemiology Section | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
| 2.4 | 0.9 | 1.5 |
| CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div> | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The need to define antigenic differences among human rotaviruses led us to cultivate directly in cell culture a variety of rotavirus strains from diverse geographical areas and populations. Selected rotavirus strains are being developed as potential vaccine candidates. Over 90 strains of human rotaviruses have been cultivated in MA104 or African green monkey kidney (AGMK) cells. Four distinct serotypes of human rotavirus were identified and compared with each other by plaque reduction or tube neutralization assay. Based upon these findings a numbering system which includes both human and animal rotaviruses was proposed. </p> | | |

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00340-03 LID

PERIOD COVERED

October 1, 1984 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Experimental Studies in Animals With Various Rotaviruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---------|--------------------------|--------------------|------------|
| PI: | Yasutaka Hoshino, D.V.M. | Visiting Associate | LID, NIAID |
| Others: | Richard G. Wyatt, M.D. | Medical Officer | LID, NIAID |
| | Jon Askaa, Ph.D., D.V.M. | Guest Worker | LID, NIAID |
| | Albert Z. Kapikian, M.D. | Head, Epid. Sect. | LID, NIAID |

COOPERATING UNITS (if any)

Ohio State University, Wooster, Ohio (E. H. Bohl, K.W. Theil, L. Saif); Plum Island Animal Center, Greenport, Long Island, N.Y. (C. A. Mebus); NINCDS, NIH, Bethesda (W.T. London); VRB, DRS, NIH, Bethesda, Maryland (James Harwell); U. of Nebraska, Lincoln, Nebraska (Alfonso Torres).

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

0.7

PROFESSIONAL

0.4

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The aims of this project are to evaluate virulence and immune response of selected human and animal rotaviruses in experimentally infected animals, and to study cross-protection between selected strains of human and animal rotaviruses. Study of potential vaccine strains in experimental animals is also useful in testing for the presence of adventitious agents.

Experimental animal systems that are currently available and that have been shown to be suitable for the study of rotavirus infection and in some cases disease, are the colostrum-deprived newborn rhesus monkey and the gnotobiotic piglet. Chimpanzees, rhesus monkeys and African green monkeys are also utilized to examine the safety of potential rotavirus vaccine preparations. Piglets were delivered by Caesarean section and maintained in plastic isolators under gnotobiotic conditions; monkeys were maintained under strict isolation in contained units.

Sera and fecal samples derived from the animal studies were analyzed for antibody and antigen by a variety of laboratory techniques. Enzyme immunoassay was used to evaluate fecal samples for antigen, while complement fixation, immune adherence hemagglutination assay and/or plaque reduction neutralization assay were used to evaluate sera and fecal samples for antibody response.

| | | |
|--|--------------------------|--|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00341-03 LID |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies of Rotaviruses in Volunteers | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) | | |
| PI: | Albert Z. Kapikian, M.D. | Head, Epid. Sect. LID, NIAID |
| Others: | Richard G. Wyatt, M.D. | Medical Officer LID, NIAID |
| | Karen Midthun, M.D. | Medical Staff Fellow LID, NIAID |
| | Yasutaka Hoshino, D.V.M. | Visiting Associate LID, NIAID |
| | Jorge Flores, M.D. | Expert, LID, NIAID |
| | Roger I. Glass, M.D. | Medical Officer LID, NIAID |
| | Robert M. Chanock, M.D. | Chief, LID LID, NIAID |
| COOPERATING UNITS (If any) Smith Kline-RIT, Rixensart, Belgium (E. D'Hont, A. Delem, S. Huygelen; Center for Vaccine Dev., U. of Md., Baltimore, Md. (M. Levine, R. Black, M.L. Clements); Flow Labs., McLean, Va. (L. Potash); University of Rochester, Rochester, N.Y. (R. Dolin). | | |
| LAB/BRANCH Laboratory of Infectious Diseases | | |
| SECTION Epidemiology Section | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
| 4.1 | 0.9 | 3.2 |
| CHECK APPROPRIATE BOXES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> A variety of human and animal rotaviruses have been grown in cell culture and are available for further study as potential vaccine candidates. In a previous study in adult volunteers immunologic correlates of resistance were defined. In the current studies these correlates (serum antibodies measured by a variety of techniques the most important being neutralization in tissue culture) were used to select volunteers for evaluation of candidate vaccine strains. Our intent was to identify rotaviruses that were capable of infecting susceptible adults and inducing immunity without the development of disease. Three candidate viruses were evaluated, the human Wa rotavirus (serotype 1) and two animal rotaviruses, the bovine UK rotaviruses and the rhesus rotavirus. The human Wa rotavirus and the rhesus rotavirus infected susceptible adult volunteers and induced a rotavirus antibody response but these viruses did not cause disease. The bovine UK rotavirus did not appear to infect adult volunteers. The rhesus rotavirus appears to be promising because it is similar by neutralization to the human serotype 3 rotavirus. Furthermore, the rhesus virus stimulated heterotypic serum rotavirus antibody responses in adult volunteers. Finally, rhesus rotavirus may prove useful as a donor of attenuating genes to reassortant viruses that bear the major neutralization protein (VP₇) of a human rotavirus belonging to serotype 1, 2, or 4. In this manner homotypic protection could be provided for each of the human serotypes. Indeed, single gene substitution reassortants are now available for each of the human serotypes. These viruses possess 10 rhesus rotavirus genes and a single human rotavirus gene, the one that codes for neutralization specificity. It is likely that such single gene substitution reassortants will induce immunity to viruses belonging to the serotype of their human rotavirus parent while at the same time they should retain the attenuation of their rhesus rotavirus parent. </p> | | |

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00342-03 LID

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Studies of Gastroenteritis Viruses by Electron Microscopy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Albert Z. Kapikian, M.D. Head, Epid. Sect. LID NIAID

COOPERATING UNITS (if any)

Dr. Nalini Singh, Children's Hospital National Medical Center, Washington, D.C.

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

0.2

PROFESSIONAL:

0.1

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The electron microscope (EM) has been a mainstay for study of fastidious gastroenteritis viruses. Although 2nd and 3rd generation tests have been developed for the detection of "Norwalk" viruses and rotaviruses, EM remains an essential tool: (1) as the "supreme court" when newer tests yield variable results; (2) in the quest for new agents of viral gastroenteritis, (3) for visualizing the site of attachment of antibody on the virion in antigen-antibody reactions; (4) for serologic studies; (5) for direct visualization of virus particles; and (6) for studying appropriate specimens derived from individuals with diseases of unknown etiology such as acquired immune deficiency syndrome (AIDS) and non-A, non-B hepatitis. As reported previously in last years annual report the site of attachment of monoclonal antibody to the gene 8 product of rhesus rotavirus (serotype 3) was visualized to be on the outer capsid of the double shelled rotavirus particle. In addition, with such ascitic monoclonal antibody, several rotaviruses belonging to the third human rotavirus serotype could be easily serotyped by IEM. The site of attachment of monoclonal antibody to the gene 6 product of rhesus rotavirus was visualized to be on particles lacking the outer capsid (rough) but not on particles with an intact outer capsid (smooth). The site of attachment of monoclonal antibody to the gene 4 product of rhesus rotavirus (the hemagglutinin) could not be detected by IEM. Finally, about 50% of the episodes of pediatric diarrhea are still without any known etiology. A major effort should be made to examine by IEM or indirect IEM such "negative" specimens in an attempt to detect new etiologic agents.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00343-03 LID

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Role of Norwalk-Like 27nm Virus Particles in Viral Gastroenteritis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Karen Midthun, M.D. Medical Staff Fellow LID, NIAID

Others: Jon Askaa, D.V.M. Guest Worker LID, NIAID
Albert Z. Kapikian, M.D. Head, Epid. Section LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

0.4

PROFESSIONAL:

0.2

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Marin County agent is a 27nm virus-like particle which was associated with two separate outbreaks of nonbacterial gastroenteritis in northern California in 1978 by L. Oshiro. The agent is morphologically similar but serologically distinct from the Norwalk, Hawaii and Snow Mountain agents as assessed by immune electron microscopy (IEM) or solid phase radioimmunoassay (RIA) antibody blocking assay. One ml of a safety tested, bacteria-free filtrate prepared from a stool specimen from one of the individuals ill during the original Marin County outbreak was administered orally to seventeen adult volunteers. None of these individuals developed definite clinical illness. Two additional volunteers later received a 20ml inoculum. One of these volunteers developed a gastrointestinal illness characterized by nausea, vomiting, diarrhea and malaise. Interestingly, this illness started five days after administration of the fecal filtrate and lasted 36-48 hours. Examination by IEM of several diarrheal stool specimens from this volunteer demonstrated a large number of 27nm particles. These particles were shown to be identical to the Marin County agent in IEM studies using acute and convalescent sera from the original outbreak. A preliminary survey of a series of gastroenteritis outbreaks using a recently developed RIA failed to implicate the Marin County agent as an important cause of epidemic gastroenteritis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00346-03 LID

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Detections and Characterization of Rotavirus by Hybridization Techniques

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

| | | | |
|---------|--------------------------|--------------------|------------|
| PI: | Jorge Flores, M.D. | Expert | LID, NIAID |
| Others: | Yasutaka Hoshino, D.V.M. | Visiting Associate | LID, NIAID |
| | Richard Wyatt, M.D. | Medical Officer | LID, NIAID |
| | Roger Glass, M.D. | Medical Officer | LID, NIAID |
| | Albert Kapikian, M.D. | Head, Epid. Sect. | LID, NIAID |

COOPERATING UNITS (if any) Irene Perez-Schael, M.Sc., "Instituto Nacional de Dermatologia, Universidad Central de Venezuela", Caracas, Venezuela

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| | | | | | |
|-----------------|-----|--------------|-----|-------|-----|
| TOTAL MAN-YEARS | 0.5 | PROFESSIONAL | 0.5 | OTHER | 0.0 |
|-----------------|-----|--------------|-----|-------|-----|

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Rotavirus single stranded (ss) RNA probes prepared by in vitro transcription of purified virions were used in a dot hybridization assay to detect the presence of rotavirus in stools and other biological materials. The assay is highly specific and sensitive and its use may facilitate epidemiological studies of rotavirus gastroenteritis. RNA transcription probes have also been employed successfully in analyzing and quantitating the degree of genetic relatedness among different rotavirus strains of human and animal origin.

Probes prepared from rotavirus cDNA cloned into pBR322 have also been used in the dot hybridization assay. They have allowed the recognition of specific rotavirus sequences corresponding to discrete genes. Application of these gene-specific probes will be useful in epidemiological studies aimed at the identification of rotavirus serotypes and other markers.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00373-02 LID

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies for Detection of Etiologic Agent(s) of AIDS by Immune Electron Microscopy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Albert Z. Kapikian, M.D

Head, Epidemiology Section LID, NIAID

COOPERATING UNITS (if any)

National Institute of Neurological and Communicative Disorders and Stroke, NIH, Bethesda, Maryland (Drs. Gravelle, London, Sever)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

<.1

PROFESSIONAL:

<0.1

OTHER

0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Acquired immune deficiency syndrome (AIDS) is a serious disease of major public health importance. Immune electron microscopy, which is the direct observation of antigen-antibody interaction, has been employed as a means for detecting the fastidious etiologic agents of several diseases such as the 27 nm Norwalk agent of acute nonbacterial gastroenteritis, and the 27nm hepatitis A virus of hepatitis. Collaborative studies using electron microscopy were carried out on simian AIDS specimens in collaboration with the NINCDS. A retrovirus-like agent was visualized by negative stain electron microscopy.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00410-01 LID

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Binding of Rotavirus to Cell Surface Receptors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Co-PI: Albert Kapikian, M.D. Head, Epidemiology Section LID, NIAID
Jon Askaa, D.V.M. Guest Worker LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

0.5

PROFESSIONAL:

0.5

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In an attempt to characterize the nature of the receptors on cell surfaces and of the viral protein involved in this initial interaction between rotavirus and cells, several approaches have been followed. (1) Enzymatic treatment of human type O erythrocytes before utilization in hemagglutination assay has resulted in the separation of rhesus and bovine (NCDV strain) rotaviruses into one group and two avian rotavirus isolates into another group with respect to their ability to agglutinate these enzymatically treated erythrocytes. (2) Non-hemagglutinating human rotaviruses have been shown to bind to human erythrocytes in a modified radioimmunoassay using the erythrocytes as solid phase. (3) Labeled rotavirus has been shown to react with membrane proteins isolated from both the microvillus of enterocytes of the small intestine of pigs as well as from human erythrocytes. (4) Rotavirus was demonstrated to bind to glycosphingolipids in a thin layer chromatography system. (5) The viral protein involved in the initial binding to MA 104 cells has in preliminary experiments been found to have a molecular weight of approximately 20,000 daltons

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00411-01 LID

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Relative Frequency of Rotavirus Subgroups 1 and 2

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Jorge Flores, M.D.

Expert

LID, NIAID

COOPERATING UNITS (if any)

Instituto Nacional de Dermatologia, Instituto Nacional de
Nutricion, Caracas, Venezuela

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

0.1

PROFESSIONAL

0.1

OTHER

0.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Monoclonal antibodies recently developed against the 42,000-dalton protein of two rotavirus strains were used in an enzyme-linked immunosorbent assay to determine the subgroup specificity of rotaviruses obtained from Venezuelan children with rotavirus gastroenteritis. Subgroup 2 rotavirus was shed by 85% of the children, whereas only 14% shed subgroup 1 rotavirus. No differences were found in the occurrence of fever and vomiting between children shedding either rotavirus subgroup, but it appeared that the syndrome tended to last longer in children shedding subgroup 2 rotavirus.

LABORATORY OF MICROBIAL IMMUNITY

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PHS-NIH
SUMMARY STATEMENT

ANNUAL REPORT OF THE LABORATORY OF MICROBIAL IMMUNITY
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
October 1, 1983 to September 30, 1984

Richard Asofsky, M.D.
Chief, Laboratory of Microbial Immunity

SUPPRESSOR AND AMPLIFIER T CELLS ACTIVATED IN RESPONSE
TO CELL ASSOCIATED ANTIBODY ON B CELLS

Both amplifier and suppressor T cells can be activated by exposure to antigen-specific B cells derived from mice exposed to a subimmunogenic dose of Type III pneumococcal polysaccharide (SSS-III). Such cells differ with respect to their Lyt phenotype, kinetics of activation, and radiation sensitivity. Specificity of the regulatory effects observed is due in whole or in part to the ability of these regulatory T cells to recognize the idiotypic determinant of B cell associated antibody specific for SSS-III. (P.J. Baker, P.W. Stashak, G. Caldes, and M. Fauntleroy, LMI, NIAID; B. Prescott, Biomedical Research Institute).

CYCLIC REGULATION OF DEVELOPMENT OF IMMUNOLOGICAL
MEMORY

Mice pretreated with a single injection of a sub-immunogenic dose of lipopolysaccharide from Escherichia coli 0113 (LPS 0113) develop immunological memory which is expressed in cyclic manner. Such memory involves the expansion of a pool of memory B cell in the absence of T cells and is under separate genetic control. The rapid decline of memory, which is responsible for the distinct peaks of memory noted in the cyclic pattern obtained, suggests the ordered expression of an inhibitory or suppressive process what may be activated in response to LPS-specific cell associated antibody or its idiotypic determinant. (P.J. Baker, P.W. Stashak, J. Hiernaux, LMI, NIAID; J.A. Rudbach, Abbott Laboratories).

SPLIT TOLERANCE IN AUTOIMMUNE MICE

Susceptibility to tolerance induction was examined in NZB and control mice by the intraperitoneal administration of monomeric BSA isolated by gel filtration, followed 14 days later by challenge with DNP-BSA in complete Freund's adjuvant, thus allowing measurement of the response to both the DNP hapten and the BSA carrier. The results indicate that exposure to soluble BSA induces tolerance

in all strains to the DNP hapten when introduced covalently coupled to BSA but not when coupled to another carrier, ovalbumin. In striking contrast, while NFS and NZW were rendered tolerant to the carrier, NZB was not. No mice gave a detectable response to soluble BSA without subsequent challenge. These results suggest that suppressor T cells can be induced normally in young NZB mice, but that B cells behave abnormally. We feel it is likely that some B cells are primed by the first exposure to BSA in such a way that they become resistant to subsequent T cell suppression and that this behavior is related to the polyclonal B cell activation of this strain. If this proves to be so, it would provide the heretofore unappreciated link between B cell hyperactivity and the resistance to tolerance. Further, if such a mechanism extended to self-antigens and if such "unsuppressible" B cells accumulated slowly, we would have a large part of the mechanism of autoimmune disease in NZB mice.

AN EARLY INTRA-ATHYMIC PRECURSOR GIVES RISE TO OTHER THYMOCYTES

A small ($\leq 5\%$) subpopulation of thymic lymphocytes from adult mice has been identified by multiparameter flow cytometric analysis. This cell (phenotype: Ly 1 dull, Ly 2⁺) has been isolated to high purity using monoclonal antibodies to cell surface antigens and a combination of cytotoxic and panning methods. Transfer of Ly 1 dull cells to irradiated Ly 1 congenic recipients shows that a portion of these cells home to the thymus, then differentiate into the 3 other populations identifiable by flow cytometric methods. An identical sequence of differentiation is seen following transfer of bone marrow cells, but with several days' delay. The sequence observed is identical to that observed in fetal ontogeny. (Drs Fowlkes, Chused, and Mathieson (NCI); Ms. Edison).

DIFFERENTIATIVE EFFECTS OF CYTOCHOLASIN B ON B CELL HYBRIDS

Cytochalasin B (CB) is a fungal product which is toxic for animal cells because it disrupts the structure of the cytoskeleton by inhibiting polymerization of actin filaments. CB has been used to study transmission of signals across membranes in circumstances in which aggregation of surface components is thought to be important; CB inhibits such aggregation. We have obtained several somatic cell hybrids between an HGPRT⁺ marked line of B lymphoma cells (M.12.4.1) from BALB/C mice and normal B lymphocytes from C57Bl/6 mice which can be induced to secrete IgM following exposure to anti IgM antibodies in vitro. About 15% of cells secrete IgM after induction; almost all cells sustain a loss of expression of surface Ia molecules after induction. We studied the effects of various amounts of CB on this differentiation. In 4 of the hybrids tested, CB had no effect on differentiation when tested alone, and failed to inhibit differentiation when tested with a suitable dose of anti IgM. The expected number of cells secreting IgM, and the expected loss of expression of Ia antigen was observed. A fifth line, TH 2.54, was induced to secretion and loss of Ia by CB alone. Effects of anti IgM, but not of CB could be blocked by inclusion an IgM paraprotein in the cultures. This study suggests that aggregation of receptors is not always needed for transduction of signals via membrane immunoglobulins.

(Drs. Hamano, (Hyogo School of Medicine) and Asofsky)

ADMINISTRATIVE

There was a significant enlargement of the group working on differentiation of B lymphocytes. Dr. Maureen Howard transferred to LMI from LI in November. Dr. Howard is a Visiting Scientist in a "tenure-track" position. Dr. David Greenblatt, a Medical Staff Fellow, and Dr. Tsuyoshi Teranishi a Visiting Associate also joined the staff during the year. Dr. Wael Jajour, a Visiting Fellow, arrived to work under the direction of Dr. Stone. Dr's Jean Langhorne and Christopher Taylor completed their Visiting Fellowships. Dr. Taylor has accepted an academic position; Dr. Langhorne has a fellowship in Freiburg, FRG beginning in November. Charles Evans, LMI's most senior technician retired, and will be replaced by a staff fellow.

| | | |
|--|---------------------|---------------------------------------|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00131-17 LMI |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Mechanism of Hypersensitivity in Inbred Histocompatible Guinea Pigs | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <div style="display: flex; justify-content: space-between;"> <div style="width: 60%;"> Sanford H. Stone Section Head Wael Jarjour Visiting Fellow Diana F. Amsbaugh Biologist M.B. Datiles C.S. Raine** </div> <div style="width: 35%; text-align: right;"> LMI, NIAID LMI, NIAID LMI, NIAID Clinical Branch, NEI Albert Einstein College of Medicine New York City, N.Y. </div> </div> Uta Traugott** | | |
| COOPERATING UNITS (if any) Clinical Branch and Laboratory of Vision Research, NEI, **Division of Neuropathology, Albert Einstein College of Medicine, New York, N.Y. | | |
| LAB/BRANCH Laboratory of Microbial Immunity | | |
| SECTION Experimental Autoimmunity Section | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MAN-YEARS 3.0 | PROFESSIONAL 2.0 | OTHER 1.0 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> We have been studying <u>autoimmune encephalomyelitis (EAE) in juvenile guinea pigs</u> which undergo a <u>chronic stage</u> after the acute phase. <u>Monoclonal anti-guinea pig T cells</u> were used to trace T lymphocytes to CNS sites. We are now using such monoclonals to separate and characterize the T cells from guinea pig spleens, lymph node and peritoneal exudate using plating, panning and cell sorter to isolate populations. The significance of the project lies in the opportunity to use monoclonal antibodies (developed at NIAID by Dr. Shevach in mice against surface antigens of lymphocytes of guinea pigs) to identify subpopulations responsible for tuberculin reactivity and induction of autoimmune diseases. </p> <p> In collaboration with researchers in the National Eye Institute, we are able to undertake genetic and immunological studies of a <u>congenital autosomal dominant cataract</u> which is a <u>useful model of congenital cataracts in man</u>. These cataracts are characterized by a <u>missing beta crystallin</u>, and preliminary studies indicate that cataractous strain 13 guinea pigs show stronger delayed type skin responses to lens proteins than normal strain 13s after immunization with strain 13 lens antigens in complete Freund's adjuvant. </p> | | |

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00134-22 LMI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Control of Immunoglobulin Synthesis in Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R. Asofsky Chief, Laboratory of Microbial Immunity LMI, NIAID

Others: C. Sulis Medical Staff Fellow LMI, NIAID
D. Greenblatt Medical Staff Fellow LMI, NIAID
B. J. Fowlkes Microbiologist LMI, NIAID
A. E. Brooks Biological Lab Technician LMI, NIAID

COOPERATING UNITS (if any)

*Hopital Broussais, Paris; **Revlon Research Groups; ***Basel Institute for Immunology; ****Hyogo University Medical School; *****Natural Immunity Section, BERM, NCI.

LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Experimental Pathology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

3.5

PROFESSIONAL:

2.5

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The effects of cytochalasin B (CB) on differentiation of B cell hybridomas following stimulation with anti IgM was studied. CB did not inhibit differentiation to secretion of IgM nor the loss of membrane-bound Ia molecules induced by anti IgM in these cells. One hybrid line was induced to secrete IgM and to reduced expression of Ia by CB alone. The results suggest that aggregation of receptors is not essential for the triggering of these cells by anti IgM.

A double adoptive transfer system was used to study induction of immunologic memory in B cells specific for DNP and in T cells specific for the protein carrier KLH. T-Helper and T-suppressor activity both developed simultaneously and their levels were sustained for many months. The cells acted asynchronously following transfer: Helper cells alone together with B cells produced sustained antibody responses; suppressor cells transferred at the same time acted to terminate the antibody responses after an early peak of synthesis of antibodies.

| OTHER PROFESSIONAL PERSONNEL | | Project Number |
|--|---------------------|--|
| (name, title, laboratory, and institute affiliation) | | Z01 AI 00134-22 LMI |
| C. Kanellopoulos-Langevin* | Investigator | Institut d'Immunobiologie Hopital, Broussais, Paris |
| K. Jin Kim** | Guest Worker | Revlon Research Group Rockville, Maryland 20205 |
| L. Lefkovitz*** | Member | Basel Institute of Immunology Basel, Switzerland |
| T. Hamano***** | Assistant Professor | Hyogo University Medical School |
| B. Mathieson***** | | Natural Immunity Section, BERMP National Cancer Institute |

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00136-12 LMI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization and Differentiation of Thymic Lymphocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: B. J. Fowlkes, Microbiologist

| | | | |
|---------|-------------------|---------------------|------------|
| Others: | Richard Asofsky | Laboratory Chief | LMI, NIAID |
| | Thomas M. Chused | Senior Investigator | LMI, NIAID |
| | Linette M. Edison | Biologist | LMI, NIAID |
| | Bonnie Mathieson | Staff Fellow | FCRF, NCI |
| | Shi Fay Cheng | Summer Student | LMI, NIAID |
| | Larry Samuelson | Staff Fellow | LI, NIAID |

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Experimental Pathology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda MD 20205

TOTAL MAN-YEARS:

1.2

PROFESSIONAL

0.2

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Examination of thymic cell types by multiparameter flow cytometric analysis of cell size and cell surface antigens has allowed the detection of seven subpopulations of mouse thymocytes. Three of the subpopulations have not been previously described. One was extensively characterized and was demonstrated to have the properties of an early precursor thymocyte. Thymic subpopulations have been isolated to higher purity using monoclonal antibodies to cell surface antigens using cytotoxic and panning methods. These subpopulations have been examined for proliferative capacity, morphology, steroid and radiation sensitivity, function, as well as differentiation potential. Isolated thymic subpopulations have been assayed for the expression of T cell antigen receptor at the mRNA and protein level. Cell fusions have been made between the isolated subpopulation of thymocyte thought to be early precursors and the BW cell line. These cell fusion products will be studied for T cell receptor gene rearrangements before and after induction of cell surface antigens with phorbol compounds and mitogens.

Ontogenetic studies on the development of the fetal thymus have demonstrated a sequence of development of various cell types with characteristics similar to thymic cell types found in adult mice.

Studies on the differentiation potential of the isolated putative early thymocytes both in vitro and in vivo have confirmed its precursor status and suggested a sequence of differentiation similar to that seen in fetal thymus. This system is being further dissected for numbers of precursors and lineage relationships. The relationship of these early thymocytes to prothymocytes originating in the bone marrow (BM) is under investigation.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00141-10 LMI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immune responses to Malaria

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---------|----------------|-----------------------|------------|
| PI: | Jean Langhorne | Visiting Fellow | LMI, NIAID |
| Others: | C. B. Evans | Laboratory Technician | LMI, NIAID |
| | R. M. Asofsky | Laboratory Chief | LMI, NIAID |
| | S. Bates | Laboratory Worker | LMI, NIAID |
| | D. Chang | Summer Student | LMI, NIAID |
| | K. J. Kim* | Guest Scientist | LMI, NIAID |
| | S. Q. Dejoy* | | |

COOPERATING UNITS (if any)

Revlon Health Care Group, Rockville, MD (*K. J. Kim); (*S. Q. Dejoy)

LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Experimental Pathology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

1.1

PROFESSIONAL

1.1

OTHER

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Plasmodium chabaudi adami in C3HeB/FeJ and C57BL/6 mice does not induce a significant malaria - specific IgG₁ response during a primary infection. This effect does not appear to be due to the fact that parasite antigens are unable to stimulate an IgG₁ response, since those antigens shared with another rodent malaria, *P. yoelii*, when presented on *P. yoelii* parasites, do induce IgG₁ antibodies. Further, a second infection with *P. chabaudi* will induce a specific IgG₁ response. The polyclonal B cell response is similar in its isotypic distribution to that of the malaria - specific response, in that IgG₁ - secreting cells are only a very minor component of the total response. Infection with *P. chabaudi* also affects the plaque - forming cell response to sheep erythrocytes. Although the total response is relatively unaffected by infection, the IgG₁ response is depressed. IgM, IgG_{2a}, IgG_{2b}, and IgG₃ are unaltered or enhanced. The mechanism of the IgG₁ regulation in this infection is currently under investigation.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00143-15 LMI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic Control of the Antibody Response to Microbial Antigens

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: P. J. Baker

Head, Microbiology and
Immunology Section

LMI, NIAID

Others: G. Caldes

Chemist

LMI, NIAID

P. W. Stashak

Microbiologist

LMI, NIAID

M. Fauntleroy

CO-OP Student

LMI, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Microbiology and Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

0.8

PROFESSIONAL:

0.3

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies conducted with F_1 and F_2 progeny of crosses between strains of inbred mice that differ greatly in their capacity to make an antibody response to Type III pneumococcal polysaccharide (SSS-III), bacterial (Leuconostoc) dextran B-1355, and lipopolysaccharide from Escherichia coli 0113 (LPS 0113) showed that multiple genes influence the magnitude of the antibody response to these antigens. Other studies using hybrids derived from crosses between C₃/HeJ, CBA/N, and RIIIS/J mice indicated that the genetic defects characteristic of these strains of mice are dissimilar and unlinked, and that autosomal, as well as X-linked, genes control the magnitude of serum IgM levels in unimmunized mice.

OTHER PROFESSIONAL PERSONNEL
(name, title, laboratory, and institute affiliation)

Project Number
Z01 AI 00143-15 LMI

J. Hiernaux*

Service de Chimi-Physique,
Campus Plaine ULB, Brussels
Belgium

B. Prescott**

Biomedical Research Institute
Rockville, Maryland 20205

J. A. Rudbach***

Abbott Laboratories
North Chicago, IL 60064

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00144-20 LMI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of the Antibody Response to Microbial Polysaccharide Antigens

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: P. J. Baker Head, Microbiology and Immunology Section LMI, NIAID

Others: P. W. Stashak Microbiologist LMI, NIAID
M. Fauntleroy CO-OP Student LMI, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Microbiology and Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

0.6

PROFESSIONAL:

0.3

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

Immunological memory to the lipopolysaccharide of Escherichia coli 0113 (LPS 0113) was generated in strains of inbred mice given a single sub-immunogenic dose of either LPS 0113 or the native protoplasmic polysaccharide of E. coli 0113 (NPP 0113). Such memory, which involved, the synthesis of only antibody of the IgM class, developed in a cyclic manner that was characteristic for the strain of mice used. It involved cell proliferation as well as differentiation and persisted for at least 25 days after priming with a single injection of a sub-immunogenic dose of LPS 0113.

OTHER PROFESSIONAL PERSONNEL
(name, title, laboratory, and institute affiliation)

J. Hiernaux*

J. A. Rudbach**

Project No.
Z01 AI 00144-20 LMI

Service de Chimie-
Physique, Campus
Plaine ULB, Brussels
Belgium

Abbott Laboratories
North Chicago, Illinois

| | | |
|---|----------------------|---------------------------------------|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00145-17 LMI |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Mode of Action of Thymus-derived (T) Suppressor and Amplifier Cells | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: P. J. Baker Head, Microbiology and Immunology Section LMI, NIAID | | |
| Others: C. E. Taylor Visiting Fellow LMI, NIAID T. E. Chused Research Investigator LMI, NIAID P. W. Stashak Microbiologist LMI, NIAID A. Brooks Biologist LMI, NIAID G. Caldes Chemist LMI, NIAID | | |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Microbial Immunity | | |
| SECTION Microbiology and Immunology Section | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MAN-YEARS: 1.8 | PROFESSIONAL: 1.3 | OTHER: 0.5 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Amplifier T cell activity can be transferred by spleen cells harvested 72 hrs after priming with Type III pneumococcal polysaccharide (SSS-III) and can be abolished by treating the transferred cells with monoclonal anti-Lyt 1, anti-Ia, or anti-Thy 1 antibodies in the presence of complement; thus, amplifier cells represent a distinct subpopulation of T cells. Amplifier T cells are radiation sensitive but insensitive to treatment with cyclophosphamide. When amplifier cells were transferred to athymic mice, the enhancement obtained was much greater than that produced in euthymic mice; this is due to the lack of suppressor T cell activity in euthymic recipients which enables amplifier T cell activity to be expressed more fully. Although the induction and activation of amplifier T cells, is antigen-specific, the product made by amplifier T cells may not be antigen-specific in its mode of action. Since amplifier T cells can be induced and activated by exposure to immune B cells, specificity is due in whole or in part to the ability of amplifier T cells - like suppressor T cells - to recognize the idiotypic determinants of B cell-associated antibody specific for SSS-III.</p> | | |

OTHER PROFESSIONAL PERSONNEL
(name, title, laboratory, and institute affiliation)

Project Number
Z01 AI 00145-17 LMI

| | |
|---------------|----------------|
| M. Fauntleroy | CO-Op Student |
| J. Chiang | Summer Student |
| B. Prescott* | |

LMI, NIAID
LMI, NIAID
Biomedical Research Institut
Rockville, MD 20852

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00146-11 LMI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (30 characters or less. Title must fit on one line between the borders.)

Immunological Studies of Components Isolated from Bacteria, Parasites and Plants

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: G. Caldes Chemist, LMI, NIAID

Others: P. J. Baker Head, Microbiology and LMI/NIAID
Immunology Section

B. Prescott*

P. W. Stashak

Microbiologist

LMI/NIAID

COOPERATING UNITS (if any)

*Biomedical Research Institute, Rockville, MD 20852

LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Microbiology and Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.0

PROFESSIONAL:

0.2

OTHER

0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Polysaccharides which cross-reacted immunologically with Type III pneumococcal polysaccharide were isolated from two fungi, *Pleurotis forniculata* and *Auricularia polytricha*. The *A. polytricha* polysaccharide was found to contain three different biuronic acids, one of which was chromatographically similar to cellobiuronic acid, the basic structural unit of Type III pneumococcal polysaccharide. It also contained glucose, galactose, xylose, and large amounts of mannose.

Chromatographically pure cellobiuronic acid was prepared from Type III pneumococcal polysaccharide by acid hydrolysis, followed by ion change and gel chromatography.

Plant polysaccharides which had been subjected to mild acid hydrolysis and chromatography with DEAE sephadex were found to be non-immunogenic in hemolysis-in-gel tests, although the acid hydrolysis and DEAE chromatography had increased their reactivity with Type III pneumococcal antiserum in Ouchterlony testing.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00153-07 LMI

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
In Vitro Responses of Human Peripheral Leukocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: R. Asofsky Chief, Laboratory of Microbial Immunity LMI, NIAID

Others: Chu Hsiao Kin Visiting Fellow LMI, NIAID
S. Shaw Senior Investigator IB, NCI

COOPERATING UNITS (if any)

None

LABORATORY
Laboratory of Microbial Immunity

SECTION
Experimental Pathology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS | PROFESSIONAL | OTHER |
|-----------------|--------------|-------|
| 1.1 | 1.1 | 0 |

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

S 11-B, a line of human B lymphocytes transformed with Epstein-Barr virus, has been studied. The cells are B lymphocytes: 100% stain for membrane immunoglobulin (mIg⁺). About 80% contain IgA, mIgA another 30-40% mIgM. None contain in IgG. Cells were separated by cell sorting and cloned. One line which is mIgA⁻ turns IgA⁺ after several generations in vitro. We hope to derive cells in the process of "switching" isotypes from these clones.

| | | |
|---|----------------------|--|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00186-11 LMI |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Pathogenesis of Autoimmunity in Inbred Strains of Mice | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) | | |
| PI: | T. M. Chused | Senior Investigator LMI, NIAID |
| Others: | Renu Lal | Visiting Fellow LMI, NIAID |
| | Kathy McCoy | Guest Worker LMI, NIAID |
| | Linette Edison | Physical Science Technician LMI, NIAID |
| | Elinor Brown | Biologist LMI, NIAID |
| | Louise Kendricks | Biologist LMI, NIAID |
| COOPERATING UNITS (if any) Phillip J. Baker, LMI, NIAID Herb Cooper, NCI | | |
| LAB/BRANCH Laboratory of Microbial Immunity | | |
| SECTION Microbiology and Immunology Section | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MAN-YEARS: 4.1 | PROFESSIONAL: 2.2 | OTHER: 1.9 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The immunologic mechanism of autoimmune disease is being investigated in the New Zealand Black strain of mice, its hybrids, and in recombinant inbred lines derived from it. The autoimmune processes which occur spontaneously in these mice closely resemble those which occur in humans. Both the cellular mechanism and its complex genetic basis are being studied using classic genetics, examination of the recombinant inbred lines, two-dimensional gel electrophoresis of isolated lymphocyte subsets and flow cytometry. | | |

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00203-05 LMI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders)

Applications of Flow Cytometry in Immunology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Thomas M. Chused Senior Investigator LMI, NIAID

| | | | |
|---------|------------------|-----------------------------|------------|
| Others: | H. A. Wilson | Guest Worker | LMI, NIAID |
| | Linette Edison | Physical Science Technician | LMI, NIAID |
| | Kathy McCoy | Guest Worker | LMI, NIAID |
| | Renu Lal | Visiting Fellow | LMI, NIAID |
| | Elinor Brown | Biologist | LMI, NIAID |
| | Louise Kendricks | Biologist | LMI, NIAID |

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Experimental Pathology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

2.6

PROFESSIONAL:

1.6

OTHER

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

Summary:

Flow cytometry, supported by advanced multiparameter data analysis, is being applied to immunologic problems in the following areas: 1) analysis of lymphocyte subset alterations in patients with AIDS, AIDS-related complex, and other immunologic disorders, 2) regulation of membrane potential by lymphocytes and neutrophils, and 3) murine T and B cell differentiation and activation.

OTHER PROFESSIONAL PERSONNEL
(Name, title, laboratory, and institute affiliation)

Project Number
Z01 AI 00203-05 LMI

B. J. Fowlkes Microbiologist
P. Baker Senior Investigator
B. Seligman
C. Brickman
J. Gallin
M. Frank
T. Folkes
K. Sell
R. Redfield
N. Warner

LMI, NIAID
LMI, NIAID

V. Chupp

A. Steinberg
R. Murphy

T. Storch

WRAIR
Becton-Dickinson
Monoclonal Antibody
Center
Becton-Dickinson
FACS Systems
NIAMDD
Carnegie-Mellon
University
NCI

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00423-01 LMI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Factor Mediated Regulation of B Cell Growth and Differentiation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Maureen Howard Visiting Scientist LMI, NIAID

Others: P. Pesavento Guest Worker LMI, NIAID
T. Teranishi Visiting Associate LMI, NIAID
D. Greenblatt Medical Staff Fellow LMI, NIAID

COOPERATING UNITS (if any)

None

LABORATORY

Laboratory of Microbial Immunity

SECTION

Experimental Pathology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

2.2

PROFESSIONAL

1.2

OTHER

2.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

B cell immune responses are regulated by a family of T cell and macrophage derived glycoproteins. Here we extend our understanding of this regulation by demonstrating the existence of two distinct T cell derived B cell growth factors which can be separated biochemically and which appear to operate on B cells at different stages of activation. We demonstrate that in contrary to previous beliefs suggesting only Lyb 5⁺ B cells are regulated by factors, in fact most if not all B cells are governed by these mediators. Our preliminary data indicate that specific induction of resting B cells via cross-linkage of membrane Ig receptors renders them apparently unresponsive to the family of non-specific maturation factors which drive resting B cells to rapid immunoglobulin synthesis. Finally, we are currently developing assays for the biochemical identification of the receptor for BCGF-1.

LABORATORY OF MOLECULAR MICROBIOLOGY
1984 Annual Report
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PHS-NIH
SUMMARY REPORT

ANNUAL REPORT OF THE LABORATORY OF MOLECULAR MICROBIOLOGY, NIAID
October 1, 1983 - September 30, 1984

Dr. Malcolm A. Martin
Chief, Laboratory of Molecular Microbiology

The Laboratory of Molecular Microbiology (LMM) applies molecular biological techniques to study the structure and regulation of prokaryotic and eukaryotic genes. Our research goals are to answer fundamental questions in microbiology by examining host cells and associated microorganisms at the molecular level. Although a major focus has been animal virus systems, bacterial and mycoplasma organisms are also investigated. Relying heavily on such biochemical techniques such as nucleic acid hybridization, restriction enzyme mapping, molecular cloning, nucleotide sequencing procedures, and the use of in vitro synthesized oligopeptides to produce specific antibodies, LMM staff members have productively investigated a variety of genes and DNA regulatory regions that play roles in the interaction of microorganisms and their host cells. In many cases newer technologies have been combined with more conventional assay systems, particularly the construction of novel and potentially biologically active DNA recombinants.

During the past year considerable progress has been made in several different areas. The focus of investigations by members of the Biochemical Virology Section has been the detailed analysis of endogenous retroviral DNAs found in the mouse and human genomes. A unique feature of infection with retroviruses is the synthesis of a DNA copy of the viral genome, a reaction catalyzed by reverse transcriptase. The newly synthesized retroviral DNA is subsequently integrated into the chromosome of the infected cell and becomes the template that is transcribed by RNA polymerase II to generate viral genomic and messenger RNAs. When mammalian reproductive tissues are infected with retroviruses, copies of viral DNA may become incorporated into, and endogenous to, the germline of that species. All vertebrates examined to date, including man, harbor multiple copies of retroviral DNA that is an integral part of their genomes. In some instances, such as the mouse, a few copies of retroviral (proviral) DNA are expressed as complete, infectious virus particles that can cause a spreading infection. In mice, the spontaneous induction of such proviruses can be correlated with the development of leukemia. In several animal species, endogenous retroviral sequences are expressed only as messenger RNA or specific viral polypeptides that can be identified immunochemically; no infectious virions are produced. In other cases, incomplete copies of proviral DNA may recombine with infectious retroviruses present in that organism to generate viral recombinants exhibiting unique biological properties. For example, in the mouse the MCF recombinant murine leukemia viruses (MuLV) are thought to be the causative agent of leukemia.

One model of retrovirus-induced leukemogenesis involves the generation of dual-tropic MCF MuLVs following recombination between spontaneously induced ecotropic MuLVs and endogenous proviral DNA segments present in mouse chromosomal DNA. Subsequent to the recombination event, the MCF MuLVs may gain entry into lymphocytes (e.g. in the thymus) and, if integration of proviral DNA

occurs at an appropriate site (perhaps in the vicinity of a putative oncogene), the animal may develop leukemia. Members of the LMM staff have previously isolated molecular clones of endogenous MuLV proviral DNA as well as proviruses associated with different classes of infectious MuLVs. During the past year, the envelope (env) gene and the long terminal repeat (LTR) of an infectious xenotropic MuLV have been sequenced and compared to analogous regions of different classes of MuLVs. Comparative analysis of these sequences revealed that the 3' half of the env gene is highly conserved among all MuLV classes; in contrast, the 5' half of this gene is quite variable and differentiates one class of MuLV from another. A comparison of the xenotropic env gene with the previously reported sequences of different MCF proviruses indicates that although they are closely related to one another (but clearly differentiable from ecotropic MuLVs), they can be distinguished. This result is certainly compatible with the unique immunochemical and viral interference properties of MCF and xenotropic MuLVs. Analysis of the env genes associated with endogenous MuLV proviruses of the mouse clearly indicates that the vast majority are closely related to MCF rather than xenotropic MuLVs. These retroviral sequences, which are present in the germline of all strains of laboratory mice, thus have the capacity to participate in recombinational events leading to the generation of disease-causing MCF MuLVs. Employing recombinant inbred strains of mice as well as somatic cell hybrids, studies were initiated during the past year to ascertain the chromosomal location of different progenitors for MCF MuLVs. One of these endogenous retroviral DNA segments could be mapped to mouse chromosome 11 near the alpha-globin gene. Interestingly, this particular endogenous proviral DNA could not be detected in mouse strains from which MCF viruses have yet to be successfully isolated.

Another example of a potentially important endogenous MuLV sequence is the Fv-4 gene which confers resistance to infection by ecotropic MuLVs. This gene, originally identified in Asiatic mice, was partially sequenced during the past year. The Fv-4 locus consists of a partial MuLV provirus containing sequences extending from the 3' terminus of the pol gene through the env and 3' LTR. The nucleotide sequence of the env region present in the cloned Fv-4 segment has features characteristic of an ecotropic rather than a xenotropic env gene. Thus, the putative Fv-4 locus represents the 3' half of an ecotropic-like MuLV segment. It is proposed that the Fv-4-mediated resistance occurs via a viral interference mechanism resulting from the production of a gp70 polypeptide that is closely related to the env gene products of most ecotropic MuLVs.

Similarly organized endogenous type C retroviral DNA has also been identified in the human genome. Using two-cycle cross-species screening of a human gene library, we have obtained more than 50 different human endogenous retroviral clones. One full-length retroviral clone was completely sequenced (8.8 kb). Alignment of the human sequence with those of Moloney (Mo) MuLV and the baboon endogenous retrovirus established that the cloned human sequence indeed represented an endogenous proviral DNA with characteristics of size, organization, and structure typical of type C retroviruses. Comparisons of the deduced amino acid homologies of the human and Mo-MuLV averaged 50-60% in the gag and pol regions; in short stretches, homology approached 80%. On the other hand, the LTRs and the env gene of the cloned human retroviral sequence shared no polynucleotide sequence homology with known infectious murine or simian retroviruses.

Distinctive termini characterized two families of human endogenous retroviral sequences. One type of repeating terminal element was associated with full-length retroviral DNA segments and resembled typical LTRs. During the past year the nucleotide sequence of five different human LTR elements was determined; they contained structural features previously described for other retroviral LTRs. These included a putative TATA box, a polyadenylation signal, and, at the termini, imperfect inverted repeats. A polypurine tract preceded the 3' LTRs, and a putative tRNA primer binding site followed the 5' LTRs. The latter sequence was not complementary to proline tRNA, the usual mammalian type C primer, but is almost a perfect match for a published rat glutamic acid tRNA. A 4 base-pair (bp) direct repeat of cellular DNA sequences flanked the retroviral DNA in the carefully studied clone. A comparison of the nucleotide sequence of five different LTRs confirmed the presence of important regulatory signals and allowed the subdivision into U3, R, and U5 regions of approximately 380, 70, and 44 bp, respectively. The U3 and R lengths are typical of type C LTRs; the U5 region, however, is significantly shorter (44 bp vs. 75 bp). The second family of human retroviral sequences contains only 4.1 kb of the gag-pol region, and no env or LTR elements. This family, like the full-length retroviral copies, was represented 35-50 times per haploid mass of human DNA. The sequence of repeats associated with this truncated class of retroviral sequences consisted of tandem arrays of an imperfect repeating unit, approximately 72-76 bp in length. The retroviral sequences present in the truncated family of human endogenous sequences could be aligned between nucleotide 1600 and nucleotide 5670 of Mo-MuLV.

Unusual retroviral structures were also identified in human genomic DNA. Several "unaffiliated" or solitary retroviral LTR elements were molecularly cloned from a human gene library. One of these clones was analyzed by nucleotide sequencing and found to be identical in length, and 95% similar in nucleotide sequence with the LTR present in one of the full-length retroviral DNAs. A 4 bp direct repeat, ACAG, immediately flanked the solitary LTR. Such a structure could represent a partial excision of a full-length provirus through homologous recombination between the LTRs or might reflect the transposition of a single LTR to a new site in human chromosomal DNA. Nucleotide sequencing of another clone generated data compatible with the complete excision of a human endogenous retroviral DNA. This elimination of the viral DNA left, as its "footprint," a 4 bp direct repeat, CCAT, at the precise site of integration of retroviral DNA in another clone. This finding is similar to the recently reported excision of a Ds element in maize, in which the direct repeat, slightly modified, was retained.

Although no infectious retrovirus has been induced from human cells, the presence of multiple copies of type C proviral DNA raises the possibility that some may be expressed in the form of RNA or polypeptide products. A number of retroviral messenger RNAs have been detected in human cells and the RNAs exhibit a degree of tissue specificity in their expression. One or both of two species of messenger RNA hybridizing to both LTR and env probes have been identified in human placenta, colon carcinoma, breast carcinoma, choriocarcinoma, promyelocytic leukemia, and transformed B cells. One mRNA is 3.0 kb in length and appears to be analogous to the "21S" spliced MuLV env messenger RNA present in infected cells. A second 1.7 kb mRNA containing LTR and putative gp70 sequences, however, is deleted in the 3' env (putative p15E) region. Such a deleted messenger RNA could potentially encode a secreted form of the gp70 protein. Two additional mRNAs of 3.6 and 2.2 kb that hybridize only to LTR

sequences and not to other human retroviral probes have also been detected; the 2.2 kb "LTR-only" RNA species is particularly abundant in human carcinoma, leukemia, sarcoma, and neuroblastoma cell lines. Partial cDNA clones of the 3.0 and 1.7 kb LTR-env-reactive mRNAs have been obtained and subjected to restriction enzyme mapping and DNA sequence analysis. A 2.2 kb cDNA clone of the 3 kb mRNA contains putative gp70 and p15E as well as 3' LTR sequences. cDNA cloning of retroviral mRNA from melanoma and colon carcinoma cells will be carried out to further analyze the structure of the "LTR-only" mRNAs.

In collaboration with Drs. John Coligan and W. Lee Maloy (Laboratory of Immunogenetics, NIAID), oligopeptides have been synthesized based on the deduced amino acid sequence of human pol and env gene segments. The oligopeptides were used to immunize rabbits and antisera obtained 15 weeks following immunization were affinity purified and used to detect specific proteins employing the Western blotting technique. A 83K polypeptide could be detected in several human cell lines, using an antibody raised against a human env oligopeptide. This env polypeptide was particularly prominent in the colon carcinoma cell line; this reaction could be blocked by prior incubation of the antiserum with the specific oligopeptide. A number of other colon carcinoma lines are being screened to evaluate the specificity of this reaction.

We are currently conducting experiments to analyze viral and cellular proteins that may regulate the expression of endogenous proviruses, particularly those that may activate endogenous LTRs. Messenger RNAs that specifically hybridize to the LTR segment associated with endogenous African green monkey (AGM) endogenous retroviruses can be detected by Northern blot hybridization. However, COS cells (AGM cells containing a defective SV40 viral genome and expressing the SV40 large T antigen) contain greatly increased levels of mRNA that hybridize to this LTR element. This finding suggests that T antigen may be stimulating transcription of the endogenous LTR promoters by cellular RNA polymerase II, perhaps as part of a generalized enhancement of the expression of a number of cellular genes. The effects on LTR transcription of SV40 T antigen mutants are currently under study.

Investigations evaluating the functional role of viral-encoded gene products that affect the tumor-inducing capacity of transformed hamster cells have also continued. Adenovirus 2 (Ad2) infection of normal hamster cells or SV40-transformed hamster cells results in an increase in their susceptibility to lysis by hamster natural killer cells and activated macrophages. The induction of increased susceptibility was associated with the accumulation of early virus-encoded proteins. Hamster cells infected with Ad12 were more resistant to lysis by hamster natural killer cells than were cells infected with Ad2. These results suggest the existence of functional differences in the T antigen proteins encoded by the early genes of the two adenovirus serotypes that may lead to the preferential survival of hamster cells infected with or transformed by the highly oncogenic Ad12. The effect of Ad2-encoded gene products on the sensitivity to lysis by nonspecific hamster effector cells was studied in cloned somatic cell hybrids formed from SV40- and Ad2-transformed hamster cells. All hybrid cells that expressed Ad2 as well as SV40 antigens were more sensitive to lysis than the parental hamster cells that were transformed by SV40 alone. To assess the possible role of early virus proteins, quantitative determinations (by gel electrophoresis of radiolabeled proteins) were made of the viral antigens expressed in nonhybrid Ad2-, SV40-transformed cells, and 14 Ad2-SV40 hybrid lines derived from these parents. A significant

correlation was found between the amount of Ad2 58K antigen expressed in the hybrid cells and their sensitivity to lysis by both hamster natural killer cells and activated macrophages. In addition, hybrid cells that express Ad2 58K proteins accumulate less SV40 T antigen than do hybrids that express only early SV40 gene products.

A major objective of the Bacterial Virulence Section continues to be an assessment of the role of plasmids and plasmid-mediated genetic traits in the ecology, taxonomic status, and pathogenic potential of streptococci. A major focus has been the study of plasmids carrying drug resistance determinants. Experiments evaluating the failure to transform competent strains of Streptococcus sanguis with certain plasmids of S. faecalis have indicated that the presence of two independent replicons in a single plasmid DNA is incompatible with replication in S. sanguis. A replication-competent plasmid isolated from S. sanguis was shown to contain only one of the two replicons originally present in the parent plasmid.

A number of S. faecalis isolates obtained from healthy farm animals and human patients exhibited the same antibiotic resistance phenotype as that associated with the well-studied streptococcal plasmid, pJH1. The presence of the erythromycin, kanamycin, and streptomycin resistance determinants of pJH1 on a single EcoRI cleavage product was confirmed by cloning this fragment in the streptococcal vector plasmid, pVA380-1. Hybrid plasmids were then used to establish the arrangement of the resistance determinants on pJH1. These same determinants are located on a single EcoRI fragment in conjugated plasmids of human and animal isolates. The cloned resistance genes are currently being used to examine the extent to which they have been disseminated among human and animal streptococcal populations. Similarly, a cloned tetracycline resistance determinant isolated from a clinical isolate of S. mutans was used to evaluate tetracycline resistance plasmids among oral streptococci. This work was carried out in collaboration with Dr. Frank Macrina (Medical College of Virginia, Richmond) and showed that the tetracycline plasmid was present in seven different oral streptococcal strains.

In another group of experiments, a BclI fragment isolated from a plasmid of S. lactis could be inserted into another streptococcal plasmid and used to transform a lac minus strain of S. sanguis. One transformant able to metabolize lactic acid was isolated; this finding suggests that streptococcal genes can now be introduced into mutant bacteria and restore the wild-type phenotype.

Anaerobic bacteria of the genus Bacteroides are inherently resistant to many commonly used antibiotics (penicillins and tetracycline). The lincosamide antibiotic clindamycin is a first-line drug used in the treatment of anaerobic infections. However, the recent emergence of clindamycin-resistant Bacteroides strains has hampered routine chemotherapy. During the past year the physical map of an 83 kb clindamycin-containing plasmid (pBI136) was prepared. The clindamycin determinant was localized to a 7.2 kb EcoRI fragment by analysis of spontaneous (< 0.1%) clindamycin-sensitive deletion derivatives of pBI136. Further study of the antibiotic resistance determinant in pBI136 revealed the existence of a 500 bp AvaI/EcoRI fragment that is also present on two other related Bacteroides plasmids. This short DNA segment may be part of a directly repeating sequence that flanks the clindamycin-resistant genes in the three plasmids and is suggestive of a transposon-like structure.

Additional efforts to develop information on the experimental pathogenicity of a new urogenital mycoplasma were carried out. Four large female chimpanzees (weighing 42-56 lb and housed at the Primate Research Institute at Holloman AFB, New Mexico) were given an intravaginal challenge of Mycoplasma genitalium with approximately 5×10^6 organisms. The urogenital tract of all four females became colonized, and followup studies detected shedding of 10^4 - 10^7 organisms for a period of 14-15 weeks postinoculation. Significant antibody to the organism was first detected in the serum of all four chimpanzees 3 weeks postchallenge and elevated antibodies (titers to 1:64-1:128) persisted for the duration of the followup (20 weeks). These observations, in combination with earlier work on urethral infections induced in male chimpanzees, and the failure to infect a variety of male monkeys (rhesus, cynomolgous) with M. genitalium, support the value of the chimpanzee model to study some important sexually transmitted disease of man.

Specific Research Accomplishments

Sequence analysis of the envelope and LTR regions of an infectious NZB proviral DNA. The env gene of a cloned infectious xenotropic MuLV provirus, originally isolated from NZB mice, was sequenced and then aligned with ecotropic and MCF MuLV sequences to show relationships among the three classes of viruses. Sequence variability (nucleotide and deduced amino acid) was confined to the 5' half of the env gene region; the 3' half of the env gene contained relatively few base substitutions. In the 5' variable region of the env gene, xenotropic and MCF proviral DNAs were closely related and clearly distinct from ecotropic proviral DNA sequences. Based on alignment of flanking deduced amino acids and lack of homology, a single region, located in the same position in the three MuLVs, was identified as a putative env binding site. (Repaske and O'Neill)

The sequence of an MuLV resistance locus has been determined. Genetic resistance to ecotropic MuLV infection occurs in some wild mice and can be correlated with a genetic locus (Fv-4) which was identified biochemically because it hybridized to an ecotropic envelope-specific MuLV probe. In collaboration with Drs. Christine Kozak and Hide Ikeda (Laboratory of Viral Diseases, NIAID), a 5.0 kb EcoRI fragment containing this putative resistance determinant was molecularly cloned and sequenced. It contained approximately 3.5 kb of MuLV sequences including the C-terminal region of pol, a complete env region, and an LTR; 3' flanking cellular sequences were also identified. The gp70 coding region was 95% homologous to the AKV ecotropic MuLV env region. One model of viral resistance predicts the expression of an ecotropic MuLV-related gp70 which can effectively compete with similar determinants located on the envelope of infectious retroviruses for cell-receptor binding sites. Expression of a gp70 mRNA which could encode an MuLV-related gp70 was identified in cells carrying the Fv-4 determinant. (Laigret and Repaske)

Retroviral constructs can be used to evaluate the host range of MuLVs. The host-range properties of MuLVs appear to be determined by the env region. Recombinant MuLVs were constructed in vitro by ligating endogenous env gene-containing segments to infectious xenotropic or MCF proviral DNAs containing gag, pol, and 5' env sequences. Analyses of the resulting recombinant viruses showed that the 5' region of the env gene contained host-range determinants. In addition, the mink cell focus-forming ability of MCF MuLVs also appears to reside in this 5' region of the env gene. (Khan and Theodore)

The LTR of the endogenous ecotropic provirus of BALB/c mice has been sequenced. A portion of the single endogenous ecotropic provirus present in the germ-line of BALB/c mice has been cloned and the 3' LTR sequenced. The structure of this LTR was compared to the sequence of LTRs associated with one of the inducible ecotropic proviruses present in AKR mice. In contrast to the BALB/c strain, nearly all AKR mice spontaneously express ecotropic murine leukemia viruses (versus only 15% of BALB/c mice) and develop disease. A two nucleotide difference within the U3 portion of the BALB/c ecotropic MuLV LTR was observed. The effect of this alteration on the biological activity of the BALB/c LTR is currently being evaluated. (Theodore and Khan)

Distinctive termini characterize two families of human endogenous retroviral sequences. Human DNA contains many copies of endogenous retroviral sequences. Characterization of molecular clones of these structures reveals the existence of two related families. One family consists of full-length (8.8 kb) proviral structures with typical LTRs. These LTR structures contain a putative TATA box, a polyadenylation signal, and, at the end, imperfect inverted repeats. A polypurine tract preceded the 3' LTRs and a putative tRNA primer binding site followed the 5' LTRs. The latter sequence is not complementary to proline tRNA, the usual mammalian-type C primer, but is an almost perfect match for a published rat glutamic acid tRNA. The second family of human endogenous retroviral sequences consists of structures which contain only 4.1 kb of gag-pol sequences, bounded by a tandem array of 72-76 bp imperfect repeats. More than 13 tandemly repeated elements were present in one of the clones analyzed. (Steele and Martin)

Unusual retroviral structures have been identified in human genomic DNA. Several clones containing an unaffiliated or solitary LTR and no other retroviral sequences were obtained following the screening of a human gene library with a human retroviral LTR probe. The LTR is more than 95% identical to other cloned LTRs and is associated with a characteristic 4 bp direct repeat that is located at its termini. This structure most likely represents a partial excision of a full-length provirus via a homologous recombination mechanism between the LTRs that are present in full-length viral DNAs. A second clone that was isolated apparently represents the complete excision of an endogenous retroviral DNA, leaving behind a tandem 4 bp target duplication, the only remnant of a previously integrated retroviral DNA. (Steele)

Retroviral and associated cellular DNA has undergone amplification. During the screening of the human gene library with a human retroviral pol probe, several clones were isolated that contained a common 5.0 kb EcoRI fragment; both viral (env and 3' LTR) and cellular sequences were located on this DNA fragment. To rule out the possibility that this apparently reiterated DNA segment represented an artefact of molecular cloning rather than amplification of a large block of DNA encompassing both viral and cellular sequences, a 700 bp cellular DNA segment, located within the putative amplified region, was used as a probe in Southern blot hybridizations of restricted human DNAs. The result of such an experiment indicated that predicted BamHI or HindIII cleavage products were recruited in the blots of human chromosomal DNA and provided support for the idea that extensive amplification had indeed occurred. (Martin)

The major class of human endogenous retroviral DNA is also present in all primates. In spite of the fact that env and LTR gene segments of full-length endogenous human retroviral DNA failed to hybridize to known infectious murine or simian retroviruses, these same DNA segments did hybridize quite efficiently to the chromosomal DNAs prepared from baboon, African green monkey, orangutan, and chimpanzee; no hybridization under relaxed conditions was observed between these segments and any other vertebrate DNAs. These results indicate that many primates (specifically, the baboon from which an infectious retrovirus has been recovered) harbor at least two major classes of endogenous retroviral sequences. One highly conserved class is present in all primates including man but is yet to be shown to be expressed as an infectious retrovirus. The second class, typified by the baboon and macaque inducible infectious retroviruses, appears to be less highly conserved and related to one another. (Martin and Rabson)

Human endogenous retroviral DNA is expressed as mRNA. A number of human tissues and cell lines contain endogenous retroviral mRNA. Human placenta contains a 3.0 kb LTR-env mRNA similar to the 21S-spliced MuLV LTR-env mRNA. This message is also present in colon carcinoma, breast carcinoma, and choriocarcinoma. A 6.8 kb mRNA that hybridizes to gag, pol, and env mRNAs is present in several hematopoietic cell lines including two immature T cell leukemias. A unique class of retroviral mRNAs that hybridizes to an LTR probe but not to gag, pol, or env probes is present in a large number of human cells. Thus, human LTRs are active as enhancers and promoters. Further studies of the in vitro and in vivo activity of human LTRs are in progress. (Rabson and Martin)

Cloning of human endogenous retroviral mRNA. Complementary DNA (cDNA) clones of two LTR and env containing placental mRNA species have been isolated and characterized by restriction enzyme analysis and nucleotide sequence analysis. A 2.2 kb cDNA clone of the 3.0 kb mRNA contains putative gp70, p15E, and 3' LTR (U3 and R) sequences. By contrast a 1.1 kb cDNA clone encompassing the 3' portion of a 1.7 kb mRNA contains putative gp70 sequences but is deleted in p15E and a portion of the U3 region. Such an mRNA could potentially encode a secreted form of gp70. (Rabson, Steele, and Martin)

The identification of retroviral proteins in mammalian cells. In collaboration with Drs. John Colligan and W. Lee Maloy (LIG, NIAID), oligonucleotides have been synthesized based on the deduced amino acid sequences of murine and human pol and env gene segments. In the mouse system the focus has been the development of antibodies that specifically recognize the unique envelope glycoproteins of xenotropic and MCF murine leukemia viruses. In the human system, attention has been directed to the env gene which appears to be expressed in a variety of human cells. An 83K polypeptide has been identified by the Western blotting technique in several human cell lines using an antibody raised against a human env oligopeptide. This retroviral gene product was particularly prominent in a colon carcinoma cell line; its reaction could be blocked by prior incubation of the antiserum with the oligopeptide that was injected into rabbits. We are currently screening a number of other colon carcinoma lines to evaluate the specificity of this reaction. (Steele, Adachi, Khan, Repaske, and Martin)

Activation of endogenous African green monkey LTRs in primate cells expressing SV40 T antigen. The expression of endogenous African green monkey (AGM) LTRs in primate cells is being studied by Northern blot hybridization. A low basal level of LTR expression is present in CV-1 (AGM kidney) cells; however, COS cells (CV-1 cells containing defective SV40 genomes and expressing SV40 T antigen) contain greatly elevated levels of LTR mRNA. Further studies of the activation of endogenous LTRs in COS cells are in progress to determine the role of SV40 T antigen in this process. (Khan and Rabson)

Human lymphotropic retroviruses have been successfully propagated. During the past year, we initiated a research program to biochemically characterize retroviruses isolated from patients with the lymphadenopathy or acute immunodeficiency syndromes. A virus stock (LAV), kindly provided by Dr. Luc Montagnier, Pasteur Institute, was titered and propagated in stimulated normal human lymphocytes. We have successfully isolated a lymphotropic retrovirus from a patient with the pre-AIDS syndrome and are using it to prepare infected cell cultures from which we will molecularly clone DNA copies of the virus. Molecular clones obtained will be used to critically evaluate the unique tropism of these agents and to generate specific viral gene products in vitro. (Martin)

Resistance to host inflammatory cell killing implies a species-specific function of early viral T protein. Infection of normal hamster cells and SV40-transformed hamster cells, both of which are highly resistant to cytolysis by natural killer cells and activated macrophages, with nononcogenic adenovirus 2 induces a 2- to 3-fold increase in their level of cytolytic susceptibility, as shown in a series of experiments carried out in collaboration with Dr. James Cook (National Jewish Hospital and Research Center, Denver, Colorado). Infection with highly oncogenic adenovirus 12 induces a significantly lower level of susceptibility to cytolysis. The induction of different levels of cytolytic susceptibility correlates with the expression of adenovirus early gene products and is not induced in cells exposed to virus inactivated by antibody or heat. The expression of cytolytic susceptibility and weak tumorigenicity in adenovirus 2-transformed cells appears to represent a more basic alteration than the expression of cytolytic resistance and aggressive tumorigenicity in cells transformed by SV40. As cells from many species transformed by SV40 are highly cytolytic and weakly tumorigenic, it is likely that SV40 genes are unable to induce the highly cytolytic, weakly tumorigenic phenotype in cells from species in which SV40 is highly oncogenic. (Lewis)

Increased levels of resistance to effector cell lysis in cells from adult-adapted, adenovirus-transformed, cell-induced hamster tumors. Adenovirus 2-transformed hamster cells produce tumors in immunoinmature newborn hamsters (less than 4 days old) but not in immunocompetent adults (greater than 5 weeks old), as shown in a series of experiments carried out in collaboration with Dr. James Cook. Such cells are relatively susceptible to lysis in vitro by hamster natural killer cells and activated hamster macrophages. By serial passage in newborns, hamster tumor lines induced by adenovirus 2-transformed cells have been adapted to grow in adult hamsters. Cells from one of these adult-adapted tumors (Ad2HE3ATL) were found to be much more resistant to lysis by hamster natural killer cells than were parental Ad2HE3 cells. (Lewis)

Dose-dependent correlation of adenovirus 2 gene expression and sensitivity to lysis by nonspecific effector cells among hybrid cells formed from SV40- and adenovirus-transformed cells. Adenovirus 2 gene expression in cloned somatic cell hybrids formed from SV40- and adenovirus 2-transformed hamster cells govern hybrid cell morphology, tumorigenicity, and sensitivity to lysis *in vitro* by hamster natural killer cells and activated macrophages in work carried out in collaboration with Drs. Kimihiro Akagi, Cephas Patch, Arthur Levine (Office of the Scientific Director, NICHD), and James Cook. All hybrid cells that express adenovirus 2 T antigens as well as SV40 T antigens were more sensitive to lysis by these effector cells; however, there were quantitative differences between the cytolytic susceptibilities of various adenovirus 2-expressing hybrids. In quantitative assays, a significant correlation was found between the amount of adenovirus 2 58K T antigen protein expressed in the hybrid cells and their sensitivity to lysis by both hamster natural killer cells and activated macrophages. In addition, hybrid cells that express adenovirus 2 58K protein accumulate less SV40 T protein than do hybrids that express only SV40 T proteins. (Lewis)

Epidemiology of β -hemolytic *Streptococcus faecalis* infections. The narrow host-range R plasmid, pJH1, from *S. faecalis* strain JH1, was shown to be a composite of at least two separable replicons. A 4.7 kb plasmid carrying the tetracycline resistance determinant of pJH1 was isolated from a strain of *Streptococcus sanguis* that had been transformed with a hybrid molecule composed of the hemolysin-bacteriocin plasmid, pJH2, and a segment of pJH1 DNA. The 4.7 kb plasmid, pDL316, consisted solely of pJH1-derived DNA. Restriction endonuclease digestion, DNA-DNA hybridization, and heteroduplex analyses indicated that pDL316 was identical, with the exception of 250 bp, to pAM Δ 1 (4.6 kb) obtained from a *Bacillus subtilis* isolate that had been transformed with the 9.0 kb *S. faecalis* plasmid, pAM Δ 1. A 26 kb plasmid, pAD2, from *S. faecalis* strain DS16 mediates resistance to erythromycin, kanamycin and streptomycin, and is completely contained in pJH1, representing the second replicon identified in this plasmid. (Banai and LeBlanc)

A comparative analysis of multiple antibiotic resistance plasmids from *Streptococcus faecalis* strains of human and animal origin. A 15 kb EcoRI fragment from pJH1, carrying the erythromycin (Em), kanamycin (Km), and streptomycin (Sm) resistance determinants, was cloned in *Streptococcus sanguis*. Subcloning experiments resulted in the separation of the individual resistance determinants and construction of a detailed restriction map of the resistance region of pJH1. These clones were used as probes to establish the presence of the same Em, Km, and Sm resistance determinants on a single EcoRI fragment in plasmids from *S. faecalis* isolates of animal origin and from human patients. A new streptococcal cloning vector (pDL406) containing the Km and Sm resistance determinants of pJH1, with a single BclI restriction site for insertional inactivation of the Sm resistance gene, was also obtained from these studies. (Rollins, Lee, and LeBlanc)

Plasmid-mediated lactose metabolism in *Streptococcus lactis*. A lactose-negative isolate of the Challis strain of *Streptococcus sanguis*, lac83, defective in the synthesis of the first two enzymes of the lactose metabolic pathway, was transformed to a lactose-positive phenotype by plasmid DNA from *S. lactis* strain ATCC11454. Plasmid DNA from this strain, when digested with certain restriction enzymes (including BclI) but not others, also transformed the lac83 strain at high frequencies, suggesting that the transforming DNA was able to integrate into the *S. sanguis* chromosome. A BclI restriction fragment

from the S. lactis plasmid DNA was ligated to the vector plasmid pDL406 and cloned into the lac83 strain. The transformant from this experiment was lactose positive, kanamycin resistant, and streptomycin sensitive (due to insertional inactivation of the streptomycin sensitive (due to insertional inactivation of the streptomycin resistance gene of the vector). (Inamine and LeBlanc)

Dissemination of a tetracycline resistance determinant among oral streptococci. A cloned tetracycline (Tc) resistance determinant from a strain of Streptococcus mutans, previously identified as a streptococcal tetM gene, was used as a probe to establish the dissemination of this gene among clinical isolates of oral streptococci. DNA from seven different strains showed strong hybridization to the probe, and two additional strains hybridized weakly. The Tc resistance element was transferred by conjugation from one of the strains in the absence of plasmid DNA. The transferred determinant occupied a chromosomal location in transconjugants and was shown to resemble the conjugative transposon, Tn916, originally detected in Streptococcus faecalis strain DS16. (LeBlanc)

Genetic and molecular analysis of antibiotic resistance plasmids in anaerobic bacteria. A restriction endonuclease map of the 83 kb Bacteroides ovatus clindamycin (Cc) resistance plasmid pBI136 was constructed using a combination of approaches. These included standard restriction digest analysis of whole plasmid or purified fragments, cloning of the EcoRI fragments, and Southern hybridization. Through the use of spontaneous Cc-sensitive deletion derivatives of pBI136, the Cc resistance determinant has been localized to a 7.2 kb EcoRI fragment. The determinant is flanked on both sides by a 1.2 kb directly repeated sequence. This transposon-like structure is almost twice the size of the Cc resistance elements associated with two other Bacteroides plasmids and comparative analysis of these three resistance determinants is underway. (Smith)

Structural analysis of non-plasmid-associated transmissible antibiotic resistance elements in anaerobic bacteria. The Bacteroides plasmid pBI136 is difficult to detect in most strain backgrounds and may be a useful tool to study the structure of non-plasmid-associated resistance determinants. Hybridization studies with whole pBI136 revealed the presence of species-specific junction fragments in total DNA preparations from strains containing this plasmid. That these fragments represent sites of integration into the host chromosome is presently under investigation. In conjunction with this study, pBI136 is being used to probe homologous sequences from Bacteroides fragilis V503, a strain which transfers Cc resistance in the absence of detectable plasmids. More than ten hybridizing sequences have been observed. Two of these were specific for the Cc resistance gene but none have homology to the direct repeat sequence associated with the Cc resistance gene on pBI136. Presently, cloned fragments from pBI136 are being used to screen a V503 genomic library for specific homologous sequences. (Smith)

Characterization of Mycoplasma genitalium. Polyclonal and monoclonal antibodies to the P1 attachment protein in virulent Mycoplasma pneumoniae strains were nonreactive with whole cell or soluble preparations from M. genitalium, or from another pathogenic mycoplasma (M. gallisepticum) with an organized terminal attachment structure. The absence of P1 protein in the new mycoplasma from the urogenital tract of man is the first evidence that more than one type of chemical moiety is involved in the attachment mechanism that mediates surface parasitism by pathogenic mycoplasmas. (Tully)

Experimental infections with Mycoplasma genitalium. Intravaginal challenge with M. genitalium induced persistent colonization of the genital tract of four female chimpanzees. Shedding of large numbers of mycoplasmas from this site over a 14-15 week period was also associated with rises in serum antibody levels to the organism in all four animals. These findings, in combination with our earlier work reporting persistent urethral infections induced in male chimpanzees, provide substantial evidence of the pathogenicity of M. genitalium for the primate urogenital tract. (Tully)

Administrative, Organizational, and Other Changes

The Laboratory of Molecular Microbiology continues to play an important role in the training of young scientists. Dr. Paul Steele completed three years of postdoctoral training with Dr. Martin as a Medical Staff Fellow and joined the Department of Clinical Pathology, Washington University, St. Louis, Missouri. Dr. Tadahito Kanda, a postdoctoral fellow with Dr. Kenneth Takemoto, completed his appointment as a Fogarty Visiting Fellow and returned to the University of Tokyo, Tokyo, Japan. Dr. Menachem Banai, who worked with Dr. Donald LeBlanc studying streptococcal plasmids as a Fogarty Visiting Fellow, returned to Jerusalem, Israel where he is employed by the International Genetic Sciences Company. Dr. Yasutaro Hamagishi, who worked in the LMM for nearly two years as a guest researcher and played a vital role in the cDNA cloning of human retroviral messenger RNAs, returned to the Central Research Laboratories in Fujisawa, Japan.

In March, 1984, Dr. Kenneth Takemoto completed 32 years as a member of the Public Health Service and retired from the NIH. His contributions in the area of papovavirology and, in particular, his counsel to the LMM staff will be sorely missed.

During the past year, two Fogarty Visiting Fellows, Drs. Akio Adachi and Frederic Laigret, began their postgraduate training in LMM. In March, 1984, Dr. John Silver, Medical Staff Fellow, transferred from the Laboratory of Viral Diseases (LVD), NIAID to LMM, NIAID. Dr. Silver will conduct a research program emphasizing hematologic and genetic studies of malignancies induced by Friend helper virus. Dr. Julia M. Inamine joined the Bacterial Virulence Section, LMM, as a Guest Researcher in October, 1983. She received a National Research Service award from the National Institute of Dental Research to conduct postdoctoral work on the genetic and molecular analysis of streptococcal strains associated with dental caries. Dr. Graham Davey, Research Officer, New Zealand Dairy Research Institute, also joined the Bacterial Virulence Section in 1984 as a Visiting Associate. He will conduct studies on streptococcal plasmid replication and compatibility and will serve as an advisor on a project involving studies of carbohydrate metabolism by streptococci.

Honors and Awards

Dr. Malcolm Martin was invited to speak at the Advanced Study Program of the Brooking Institution for a conference entitled, "Outlook on Issues in Science and Technology" in November, 1983. In June, 1984 Dr. Martin presented lectures at "International Conference on RNA Tumor Viruses in Human Cancer," organized by the AMC Cancer Research Center in Denver, Colorado and at a workshop, "Genomic Rearrangements and the Origin of Neoplasia," in Sils Maria, Switzerland. In July, 1984 Dr. Martin participated in the workshop, "Expression of Endogenous Retrovirus-Like Sequences in Vertebrates" held in Wurzburg, Germany. On July 31, 1984 he spoke at the "Cell Substrate Workshop" held by the FDA to evaluate contaminating DNA in pharmaceutical products. Dr. Martin continues to serve on the Recombinant DNA Executive Committee, NIH and is the Chairman of the Promotion and Tenure Committee, NIAID.

Dr. Donald LeBlanc was appointed Planning Committee for an International Symposium on "The Appropriate Use of Antibiotics," sponsored by the Fogarty International Center in December, 1983. He was also reappointed to the Program Advisory Group for contracts related to the use of antibiotics in animal feeds, Division of Veterinary Medicine, FDA, in March, 1984. Dr. LeBlanc was invited to lecture at the Department of Microbiology, School of Agriculture, Cornell University, in October, 1983.

Dr. Joseph Tully continues being Associate Editor, International Journal of Systematic Bacteriology, and is a member, International Subcommittee on the Taxonomy of Mollicutes, that met in Israel during June, 1984. He is a member of the Board for International Research Program on Comparative Mycoplasmaology, the J. Roger Porter Award Committee and Selection Committee for Wellcome Visiting Professorships in Microbiology, and the Review Panel of the International Nomenclature of Diseases. Dr. Tully was an invited co-convenor of a symposium entitled, "Cell Biology of Spiroplasmas," that was a component of the International Organization for Mycoplasmaology, Fifth Congress, held in June, 1984, in Jerusalem, Israel. He was also an invited speaker at the symposium on "Taxonomy and Pathogenicity of Mycoplasmas" sponsored by the Society for General Microbiology, held in Sheffield, England, in September, 1984.

Dr. Arifa Khan was invited to participate in a workshop entitled, "Endogenous Retrovirus-Like Sequences" held in Wurzburg, Germany, in July, 1984. While in Germany, Dr. Khan was asked to give a lecture at the Institut für Immunbiologie, Freiburg, Germany. Dr. Khan was also invited to lecture at the Institute of Physical and Chemical Research, Wako, Japan following her participation at the International Virology Congress in September, 1984.

Dr. Arnold Rabson was an invited speaker at the Department of Microbiology, University of Pennsylvania, Department of Pathology, George Washington University, and the Departments of Laboratory Medicine and Pathology, University of North Carolina, during the past year.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00006-13 LMM

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders)

Competence Development and Genetic Exchange Mechanisms Among Streptococci

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Jon M. Ranhand

Senior Scientist

LMM, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Bacterial Virulence Section

INSTITUTE AND LOCATION

NIAID, NIH, Frederick, Maryland 21701

TOTAL MAN-YEARS

1.0

PROFESSIONAL

1.0

OTHER

0

CHECK APPROPRIATE BOX(ES)



(a) Human subjects



(b) Human tissues



(c) Neither

☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided)

Last year I constructed a restriction enzyme map of a chimeric plasmid designated pJR4 that was derived from the ligation of the EcoRI "B" fragments from pAM alpha 1 and pAM beta 1. pJR4 is a tetracycline resistance plasmid and is now officially designated as pRAN4. Its molecular size has been revised downward from 8.3 kilobase pairs (kb) to 7.8 kb. This year I examined other putative tetracycline resistance clones for plasmids and found the following. There are to date four classes of plasmids that came out of the original ligated mixture that was introduced by transformation into Streptococcus sanguis, strain Wicky. The first most prevalent class contains plasmids with two EcoRI restriction sites and are composed entirely of the EcoRI B fragments of both pAM alpha 1 (3 kb) and pAM beta 1 (5.3 kb); an example of this class is pRAN20 (8.3 kb). The second class also contains two EcoRI sites but suffers a deletion in the pAM alpha 1 part of the molecule. An example of this class is pRAN5 (7.9 kb). The third class of plasmids, which also suffers deletions, has single EcoRI sites. Examples of this class are pRAN4 and pRAN1 (7.4 kb). The fourth class of plasmids consists only of the EcoRI B fragment from pAM alpha 1. It also contains a single EcoRI site. Examples are pRAN16, pRAN17, and pRAN24 (all are 3 kb). The first three classes of plasmids are of interest because each contains all or part of separate replicons, namely, those derived from pAM alpha 1 and pAM beta 1. The fourth class contains only the pAM alpha 1 replicon. I am currently trying to ascertain which replicon is the functional one in the first three classes of molecules. I also modified a plasmid purification method that yields native plasmid molecules that are easily cut with a variety of restriction enzymes. The method, in general, deproteinizes total nucleic acids in cell lysates and then denatures chromosomal DNA by use of a buffer at pH 12.3. The method can be used for screening clones for plasmids as well as preparing plasmids in large amounts.

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| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00018-17 LMM |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biological and biochemical Characterization of Human Papovaviruses | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) | | |
| PI: | Kenneth K. Takemoto | Chief, Viral Biology Section LMM, NIAID |
| Others: | Tadahito Kanda | Visiting Fellow LMM, NIAID |
| | | |
| COOPERATING UNITS (if any) | | |
| LAB/BRANCH Laboratory of Molecular Microbiology | | |
| SECTION Viral Biology | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MAN-YEARS | PROFESSIONAL | OTHER |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <div style="height: 400px; border: 1px solid black; padding: 10px;"> <p>This project has been terminated.</p> </div> | | |

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| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER ZOI AI 00027-17 LMM |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Basic Studies of Mycoplasmas | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) PI: Joseph G. Tully Chief, Mycoplasma Section LMM, NIAID Others: David L. Rose Research Microbiologist LMM, NIAID | | |
| COOPERATING UNITS (if any) Clin. Res. Center, London, England (D. Taylor-Robinson); Univ. Southern Alabama, Mobile, Alabama (F. Bastian); Univ. Texas, San Antonio, Texas (J.B. Baseman); Primate Research Institute, Holloman Air Force Base, New Mexico (C. Graham). | | |
| LAB/BRANCH Laboratory of Molecular Microbiology | | |
| SECTION Mycoplasma Section | | |
| INSTITUTE AND LOCATION NIAID, NIH, Frederick, Maryland 21701 | | |
| TOTAL MAN-YEARS 3.0 | PROFESSIONAL 1.0 | OTHER: 2.0 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) <p> These efforts cover both basic and applied aspects of mycoplasmas and related wall-free prokaryotes (mollicutes), including their molecular biology, membrane and cellular components involved in attachment, virulence, or immunological inter-relationships, and their possible role in human diseases or diseases of uncertain etiology. Current work is directed to further characterization of a newly discovered mycoplasma (<u>Mycoplasma genitalium</u>) in the urogenital tract of patients with nongonococcal urethritis. The organisms share some partial antigenic relationship to another pathogenic mycoplasma (<u>M. pneumoniae</u>), and this relationship was thought to be mediated by similarities in surface components on the unique terminal attachment structure found in both organisms. Recently, we have shown that PI attachment protein present in virulent strains of <u>M. pneumoniae</u> is not present in virulent <u>M. genitalium</u> strains or in other mycoplasmas with known attachment organelles (<u>M. gallisepticum</u>), although a number of cross-reactive but distinct proteins were apparent between <u>M. genitalium</u> and <u>M. pneumoniae</u> strains. This information indicates, for the first time, that more than one type of attachment moiety is involved in the surface parasitism by mycoplasmas and suggests that these cross-reactive proteins are responsible for the observed serological interactions between these two mycoplasmas. Additional experimental pathogenicity studies in female chimpanzees provided further evidence for the virulence of <u>M. genitalium</u>. Four animals challenged intravaginally with the organism shed large numbers of mycoplasma from the vagina for 14 weeks. These observations in combination with earlier studies on urethral infections induced by <u>M. genitalium</u> in male chimpanzees, and our failure to infect a variety of male monkeys with this organism, supports the value of the chimpanzee model to study some sexually transmitted diseases of man. </p> | | |

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| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00190-06 LMM |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) The Molecular Genetics of Eukaryotic Cells and Their Viruses | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) | | |
| PI: | Malcolm A. Martin | Chief LMM, NIAID |
| Others: | Akio Adachi | Visiting Fellow LMM, NIAID |
| | Paul E. Steele | Medical Staff Fellow LMM, NIAID |
| COOPERATING UNITS (if any) Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, National Institutes of Health (Drs. John Coligan and Lee Maloy) | | |
| LAB/BRANCH Laboratory of Molecular Microbiology | | |
| SECTION Biochemical Virology Section | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MAN-YEARS 4.65 | PROFESSIONAL: 1.15 | OTHER: 3.50 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) <p>Studies carried out under this project focus on the structure and function of mammalian retroviruses. A perplexing feature of the envelope gene associated with human endogenous retroviral DNA has been its unrelatedness to known infectious mammalian retroviruses. These cloned <u>env</u> segments fail to hybridize to clones of baboon endogenous virus or Moloney leukemia virus under any hybridization condition. During the past year, however, we have demonstrated that the human <u>env</u> DNA segment reacts strongly with orangutan, baboon, chimpanzee, African green monkey, and, of course, human chromosomal DNAs; no hybridization could be detected with mouse DNA. This result indicates that baboon genomic DNA, for instance, contains at least two major classes of endogenous sequences: one that gives rise to the infectious BaEV and a second that is very closely related to human endogenous retroviral segments. Based on this result, we have rescreened a human gene library under selective hybridization conditions and have identified several clones that belong to a second major class of endogenous retroviruses. Work is in progress to establish the structural and functional relationships of the two classes of endogenous human retroviral sequences.</p> <p>A series of oligopeptides whose amino acid sequence was deduced from the primary nucleotide sequence of both murine and human retroviral DNAs was synthesized in collaboration with Drs. Coligan and Maloy (LIG, NIAID). Based on our ability to detect retroviral-specific RNA in human placentas, colon carcinoma, and breast carcinoma cells, we have raised antibody against synthetic oligopeptides whose primary sequence was deduced from different portions of the human retroviral <u>env</u> region. A 83K polypeptide has been identified in several different human cell lines; this putative retroviral gene product is particularly prominent in colon carcinoma cells. The reactivity of the rabbit antibody with the human <u>env</u> polypeptide can be blocked by prior incubation with the human retroviral envelope oligopeptide.</p> | | |

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| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER ZOI AI 00218-03 LMM |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders) Biochemical and Chemical Studies on Retroviral DNA | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) | | |
| PI: | Roy Repaske | Research Chemist LMM, NIAID |
| Others: | Raymond O'Neill | Chemist LMM, NIAID |
| | Frederic Laigret | Visiting Fellow LMM, NIAID |
| | Christine Kozak | Senior Staff Fellow LVD, NIAID |
| | H. Ikeda | Visiting Fellow LVD, NIAID |
| | Janet Hartley | Res. Microbiologist LVD, NIAID |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Molecular Microbiology | | |
| SECTION Biochemical Virology Section | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
| 3.0 | 2.0 | 1.0 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The nature of retroviral-like sequences in normal human genomic DNA and their relationship to other endogenous retroviral sequences have been studied by 1) DNA sequencing, 2) computer-assisted analysis of nucleotide and deduced amino acid sequences and 3) comparative analysis of different retroviral sequences. A full-length clone (8.8 kb) of an endogenous human retrovirus was sequenced and found to have all of the structural features and regulatory signals of a typical retrovirus. Nucleotide deletions and termination signals render it nonfunctional. Preliminary sequencing of a new African green monkey DNA clone suggests that a virus related to the human endogenous retrovirus has also infected monkeys. This retroviral DNA is not related to the HTLV lymphotropic class of human retroviruses.</p> <p>We reported the first successful cloning of an infectious xenotropic murine leukemia virus (MuLV). The retroviral <u>env</u> gene determines host range, patterns of viral interference, and specific antigenicities. We have sequenced the <u>env</u> gene and associated regions (3 kb) of the NZB xenotropic provirus and compared its nucleotide sequence with other classes of MuLVs. Analyses indicated a corresponding region in each type of MuLV could encode for an oligopeptide that may be responsible for the biological properties noted above. It should be possible to test the model by constructing a recombinant in which the putative host range determinant sequence is replaced by the corresponding sequence from another type of provirus.</p> <p>Certain wild mice have a genetically determined resistance (Fv-4) to infection by ecotropic MuLVs. We have molecularly cloned and sequenced an endogenous <u>pol-env-LTR</u> segment that appears to be responsible for this phenotype. Nucleotide sequence analysis indicates that the Fv-4 determinant has features characteristic of an <u>ecotropic rather than a xenotropic env gene</u>.</p> | | |

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| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00219-03 LMM |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders) Molecular and Genetic Analysis of Streptococci | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) | | |
| PI: | Donald J. LeBlanc | Acting Chief, Bacterial Virulence Section LMM, NIAID |
| Others: | Menachem Banai Julia M. Inamine Larry D. Rollins Linda N. Lee | Visiting Fellow Guest Researcher (Postdoctoral Fellow, NIDR) Guest Researcher Chemist LMM, NIAID LMM, NIAID LMM, NIAID LMM, NIAID |
| COOPERATING UNITS (if any) Department of Microbiology and Immunology, Virginia Commonwealth University, Richmond, Virginia (F. Macrina). | | |
| LAB/BRANCH Laboratory of Molecular Microbiology | | |
| SECTION Bacterial Virulence Section | | |
| INSTITUTE AND LOCATION NIAID, NIH, Frederick, Maryland 21701 | | |
| TOTAL MAN-YEARS 2.4 | PROFESSIONAL 1.4 | OTHER 1.0 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) <p>The narrow host-range R plasmid, pJH1, from <u>Streptococcus faecalis</u> strain JH1, was shown to be a composite of at least two separable replicons. A 4.7 kb plasmid carrying the tetracycline (Tc) resistance determinant of pJH1 was isolated from a strain of <u>Streptococcus sanguis</u> that had been transformed with a hybrid molecule composed of pJH2 and a segment of pJH1 DNA. The 4.7 kb plasmid, pDL316, consisted solely of pJH1-derived DNA and was virtually identical to pAMΔ1, one of two replicons comprising the Tc resistance plasmid, pAM1, of <u>S. faecalis</u> strain DS5. Plasmid pAD2, a 26 kb R plasmid from <u>S. faecalis</u> strain DS16, is completely contained in pJH1 and represents the second replicon identified in this plasmid. The erythromycin (Em), kanamycin (Km), and streptomycin (Sm) resistance determinants of pJH1 reside on a 15 kb EcoRI fragment which has been cloned in <u>S. sanguis</u>. Subcloning experiments led to the separation of the individual resistance determinants and to the construction of a detailed restriction endonuclease map of the resistance region of pJH1 and pAD2. These clones were used as probes to demonstrate the presence of these Em, Km, and Sm resistance determinants on single EcoRI fragments in plasmids from seven <u>S. faecalis</u> isolates of animal origin, and two from human patients. One of the hybrid plasmids obtained from the cloning experiments contained the Km and Sm resistance determinants of pJH1. Insertion of DNA into the single <u>BclI</u> restriction site of this plasmid (pDL406) results in the inactivation of the Sm resistance gene, a property that was exploited in the cloning of plasmid-associated lactose metabolic genes from a strain of <u>Streptococcus lactis</u>. A cloned Tc resistance determinant from a strain of <u>Streptococcus mutans</u>, previously identified as a streptococcal <u>tetM</u> gene, was used as a probe to establish the dissemination of this gene among clinical isolates of oral streptococci. The Tc resistance element was transferred by conjugation from one of these isolates in the absence of plasmid DNA and was shown to resemble the conjugative transposon, Tn916, from <u>S. faecalis</u> DS16.</p> | | |

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00220-02 LMM

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Function of the Oncogene Products of Polyomavirus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Dr. Yoshiaki Ito Visiting Scientist LMM, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Biochemical Virology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been terminated.

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| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00222-03 LMM |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Characterization of Endogenous Ecotropic and Xenotropic Murine Leukemia Viruses | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Theodore S. Theodore Research Microbiologist LMM, NIAID | | |
| Others: Malcolm A. Martin Chief LMM, NIAID Arifa S. Khan Senior Staff Fellow LMM, NIAID Janet Hartley Res. Microbiologist LVD, NIAID | | |
| COOPERATING UNITS (if any) University of Connecticut, Storrs, Connecticut (Torg Frederickson) | | |
| LAB/BRANCH Laboratory of Molecular Microbiology | | |
| SECTION Biochemical Virology Section | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MAN-YEARS 1.05 | PROFESSIONAL 1.05 | OTHER 0.0 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Endogenous ecotropic viruses are known to be differentially expressed in various inbred mouse strains. AKR mice, which may harbor up to five copies of such endogenous ecotropic proviral DNAs, produce virus shortly after birth and most animals develop leukemia within a year. In contrast, BALB/c mice have only a single endogenous ecotropic provirus and only a few animals (10-15%) develop leukemia late in life. To evaluate why BALB/c mice inefficiently express ecotropic proviruses, we cloned and sequenced the LTR of one of the BALB/c endogenous provirus and compared it to the LTR of inducible ecotropic proviruses isolated from AKR mice. Nucleotide sequence analysis revealed two base differences in the U3 region of the two LTRs.</p> <p>To study the relationship between dual-tropic MuLVs and leukemogenesis, we have cloned two dual-tropic MuLVs, MCF-13 (thymotropic strain) and MCF-111 (non-thymotropic strain), from chronically infected mink cells. Three MCF-13 clones were isolated.</p> <p>Transfection of cloned DNAs onto mink cells resulted in the production of infectious progeny virions. The cloning of these two types of recombinant MCF MuLVs will permit the molecular dissection of viral genes in experiments evaluating thymotropism, mink cell focus formation, and leukemogenesis. These cloned DNAs will be studied in combination with other relevant MuLV clones such as ecotropic, xenotropic, and endogenous proviral DNAs previously isolated by us from infected or normal mouse cells.</p> | | |

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| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER ZO1 AI 00353-02 LMM |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular Biology and Biochemical Structure of Endogenous Proviruses of Mice | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) | | |
| PI: Arifa S. Khan | Senior Staff Fellow | LMM, NIAID |
| Others: Theodore Theodore Arnold B. Rabson Janet W. Hartley | Research Microbiologist Medical Staff Fellow Chief, Viral Oncology Section Microbiologist | LMM, NIAID LMM, NIAID LVD, NIAID LVD, NIAID |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Molecular Microbiology | | |
| SECTION Biochemical Virology Section | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MAN-YEARS: 3.10 | PROFESSIONAL: 1.10 | OTHER: 2.00 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The mouse genome contains 30-50 copies of endogenous murine leukemia viral (MuLV)-related sequences. Only a few of these represent inducible viral loci. We previously isolated several endogenous MuLV proviruses from BALB/c and AKR/J mouse DNA. Molecular and biochemical characterization of the endogenous MuLV DNAs indicated that about 50% of the cloned DNAs were related to known MuLV proviruses. DNAs belonging to this class could be distinguished from known infectious MuLV DNAs by the presence of a 190 bp insertion in the U3 LTR region, unique restriction sites, and env sequences which were highly related to recombinant mink cell focus-forming (MCF) MuLVs. Chromosomal mapping studies allowed us to locate one of these endogenous MuLV DNAs containing an MCF-type env gene on chromosome 11 in the vicinity of the alpha-hemoglobin gene. This endogenous provirus was absent in mouse strains from which MCF viruses have not been successfully isolated. Analysis of recombinant viruses constructed <u>in vitro</u> demonstrated that the host-range property of MCF MuLVs is due to determinants located in the 5' env. Furthermore, the mink cell focus-inducing ability unique to MCF viruses also resides in the same region of the env gene. A novel class of endogenous MuLV DNAs was identified, distinguishable from other MuLV proviruses based upon absence of restriction sites characteristic of known MuLV proviral DNAs and lack of reactivity to a generalized MuLV LTR DNA probe. Nucleotide sequence analysis indicated that MuLV DNAs comprising this new class had a retroviral-like genomic structure and were distantly related to known MuLV proviruses in their sequence. We are currently studying sequences located at the viral termini of these MuLV LTR-negative DNAs in an effort to identify regulatory elements which may represent potential new LTRs associated with these proviral DNAs. </p> <p> Studies on the regulation of endogenous African green monkey (AGM) LTR expression suggested that the T antigen of SV40 virus may enhance the expression of the LTRs. COS cells that express T Ag contained greatly elevated levels of AGM LTR mRNA when compared to levels present in the parental CV-1 cells which lack T antigen. Further studies of the transcriptional regulation of endogenous LTRs are in progress. </p> | | |

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| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER ZO1 AI 00388-01 LMM |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Functional Analysis of Mammalian Endogenous Retroviral Sequences | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) | | |
| PI: Others: | Arnold B. Rabson Malcolm A. Martin Arifa S. Khan Paul E. Steele | Medical Staff Fellow Chief Senior Staff Fellow Medical Staff Fellow LMM, NIAID LMM, NIAID LMM, NIAID LMM, NIAID |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Molecular Microbiology | | |
| SECTION Biochemical Virology Section | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MAN-YEARS 1.90 | PROFESSIONAL 1.40 | OTHER .50 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The primary goal of the project is the analysis of the expression and regulation of endogenous retroviral sequences. Studies include a survey of the distribution of human retroviral mRNAs, characterization of their structure, analysis of their regulation, and ultimately investigation into potential functions of retroviral sequences in normal and malignant tissues. Subgenomic probes derived from the LTR, <u>gag</u>, <u>pol</u>, and <u>env</u> regions of molecularly cloned human, monkey, and murine endogenous retroviral DNA have been used in Northern blot hybridizations to analyze retroviral mRNA expression in these species.</p> <p>Human endogenous retroviral mRNA has been detected in a wide variety of human tissues: LTR and <u>env</u> hybridizing mRNAs in placenta, colon carcinoma, breast carcinoma and choriocarcinoma cells; <u>gag</u>, <u>pol</u>, and <u>env</u> mRNA in a number of hematopoietic cell lines, including two immature T cell leukemias and frequent "LTR-only" mRNAs in a large number of malignant cell lines as well as normal spleen and appendiceal carcinoma tissue. cDNA clones of human placental retroviral mRNAs have been obtained and a cDNA clone of a 1.7 kb LTR-<u>env</u> mRNA has been found to contain putative gp70 and LTR sequences but is lacking the p15E region. A cDNA clone of a second 3.0 kb placental LTR <u>env</u> mRNA contains putative p15E sequences as well as gp70 and LTR sequences.</p> <p>The structure of aberrantly sized retroviral mRNAs present in normal mouse tissues has been analyzed by Northern blot hybridization. In addition to the expected 8.4 and 3.0 kb retroviral mRNAs, additional species of 7.0, 6.2, 5.8, 3.8, 1.8, and 1.3 kb have been detected and shown to contain variable deletions, particularly of the <u>env</u> region.</p> | | |

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00395-01 LMM

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic and Molecular Analysis of Anaerobic Bacteria Indigenous to Humans

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C. Jeffrey Smith

Visiting Fellow

LMM, NIAID

COOPERATING UNITS (if any)

Department of Microbiology and Immunology, Virginia Commonwealth University,
Richmond, Virginia (F.L. Macrina)

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Bacterial Virulence Section

INSTITUTE AND LOCATION

NIAID, NIH, Frederick, Maryland 21701

TOTAL MAN-YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Anaerobic bacteria of the genus Bacteroides are inherently resistant to most commonly used antibiotics (penicillin, aminoglycosides). The lincosamide antibiotic clindamycin (Cc) is routinely used to control Bacteroides infections but resistant strains are being isolated more frequently. Studies on the genetic and molecular basis of Cc resistance in Bacteroides were performed.

A Bacteroides ovatus clinical isolate transferred Cc resistance by a conjugation-like mechanism and was found to harbor two plasmid species, pBI136 (83 kilobases [kb]) and pBI106 (44 kb). Cc resistance was associated only with pBI136 but pBI106 was 90% homologous to a previously described Cc resistance plasmid (pBF4) with the exception of the specific pBF4 region implicated in the resistance. Restriction endonuclease maps of pBI136 and pBI106 were constructed and the Cc resistance determinant on pBI136 was localized. This region of pBI136 was shown to have homology to the Cc resistance determinant of pBF4.

The pBI136 Cc resistance determinant has been cloned and its structure studied. Results indicate a transposon-like structure with the Cc resistance gene flanked by directly repeated sequences of 1.2 kb. Comparative studies with two other Bacteroides strains have been initiated.

Transmissible Cc resistance among Bacteroides spp. also occurs by a process which does not involve detectable extrachromosomal elements. The plasmid pBI136 is extremely difficult to detect and may provide insight into the mechanism of the non-plasmid associated transmissible Cc resistance. In collaboration with Dr. Macrina, pBI136 is being used to probe the molecular structure of one such model system in Bacteroides fragilis strain V503.

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| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00399-C1 INT |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Structural Studies of Endogenous Primate Retroviral Sequences | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) | | |
| PI: Malcolm A. Martin Chief | | IMM, NIAID |
| Others: Paul E. Steele Medical Staff Fellow | | IMM, NIAID |
| COOPERATING UNITS (if any) Laboratory of Viral Diseases, National Cancer Institute, NTP, Frederick Cancer Research Facility, Frederick, Maryland (Stephen O'Brien) | | |
| LAB/BRANCH Laboratory of Molecular Microbiology | | |
| SECTION Biochemical Virology Section | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MAN-YEARS 2.95 | PROFESSIONAL .70 | OTHER: 2.25 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Using techniques of molecular cloning, nucleotide acid hybridization, and nucleotide sequencing, more than 50 retrovirus-like DNA segments have been cloned from a human gene library. Work carried on during the past year indicates that the major class of endogenous, retrovirus-like DNA can be divided into two closely related families each of which is represented 35-50 times per haploid mass of human DNA. One family consists of typical full-length retroviral sequences including segments that encode <u>gag</u>, <u>pol</u>, and <u>env</u> gene products; its termini contain cross-reactive 500 bp elements that have features characteristic of retroviral LTRs. Each LTR contains an imperfect inverted complementary repeat (6 or 7 out of 10 match), a putative TATA box, a typical polyadenylation signal, a possible CCAAT sequence, and a 13 bp polypurine tract. A potential tRNA primer binding site that follows the 5' LTR is a 16/18 or a 17/18 match with the 3' end of a rat glutamic acid tRNA. The second family of endogenous retroviral sequences contains 4.1 kb of <u>gag</u> and <u>pol</u> sequences that are very similar to those present in the full-length clones. The truncated family, however, is not bounded by LTRs nor does it contain any <u>env</u> sequences. Instead, the retroviral sequences are flanked by a tandem array of imperfect repeats 72-76 bp in length.</p> <p>Further analysis of several human retroviral clones indicated that large blocks of DNA involving both viral and flanking cellular DNA sequences have been amplified subsequent to the integration of viral DNA into the human chromosome during the early phases of viral infection. Southern blot hybridization was used, in one instance, to demonstrate that at least one amplification event occurred prior to the divergence of man and chimpanzee since similar recruited restriction enzyme fragments could be detected in the DNAs of these two primates. Other hybridization experiments employing somatic cell hybrids and carried out in collaboration with Dr. Stephen O'Brien (NCI), indicated that copies of retroviral segments were present on several different human chromosomes.</p> | | |

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 AI 00415-01 LMM

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

The Molecular Biology of Retroviruses Associated with AIDS

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Malcolm A. Martin

Chief

LMM, NIAID

COOPERATING UNITS (if any)

Laboratory of Viral Diseases, NIAID; Laboratory of Immunogenetics, NIAID

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Biochemical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

10

PROFESSIONAL:

10

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

During the past year a research program was initiated to biochemically characterize retroviruses isolated from patients with the lymphadenopathy or the acute immunodeficiency syndromes (AIDS). A virus stock (LAV) and detailed protocols for stimulating human lymphocytes and propagating lymphotropic human retroviruses were kindly provided by Dr. Luc Montagnier, Pasteur Institute, Paris. The initial virus stocks were titrated and propagated in stimulated normal human lymphocytes and lymphotropic retrovirus pools were prepared. We have been successful in isolating a lymphotropic retrovirus from a patient with the pre-AIDS syndrome and are preparing high-titer pools of this new inoculum. In collaboration with Dr. Tom Folkes (LIG, NIAID) lymphotropic retrovirus stocks have been propagated in a continuous cell line (CEM) yielding virus titers comparable to those obtained with stimulated normal human lymphocytes.

We are currently in the process of molecular cloning lymphotropic retrovirus proviral DNAs from infected cells. Molecular clones obtained will be used in two types of experiments. Constructs will be made utilizing the LTR and other gene segments to introduce a variety of DNAs into subsets of T-helper lymphocytes. *In vitro* mutagenesis will be carried out to ascertain the principal determinants of host range. In a second group of experiments lymphotropic retroviral gene segments will be inserted into a variety of expression vector systems to elicit the synthesis of novel viral proteins.



LABORATORY OF PARASITIC DISEASES

1983 ANNUAL REPORT

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Laboratory of Parasitic Diseases

National Institute of Allergy and Infectious Diseases

Summary - October 1, 1983 - September 30, 1984

INTRODUCTION

One move leads to another - now that the Malaria Section has rejoined the rest of the laboratory in Building 5, we start planning for a move of entire LPD to a still to be renovated Building 4 a few years hence! Our professional personnel are also on the move: Staff Fellows Stephanie James to a job at George Washington University, Frank Collins to the CDC, Peter David to a position in France and "Chip" Sheppard with a commercial firm in California. Dr. von Lichtenberg's Fogarty Scholarship was all too brief, but we look forward to his return next year. Prof. Paul Basch returned to Stanford after spending his sabbatical year part time with us as a guest worker. Among the Guest Researchers the Drs. Teixeira returned to Brazil and Ingeborg Perez will start a doctorate program in Mexico City. Visiting Fellow Ramesh Paranjape returned to Madras, India. We will miss the cheery greetings of a veteran Lab Tech, Tommy Halleck, who retired. Although Medical Staff Fellow "Skip" Francis officially left LPD he will be working with Dr. Sell's group on AIDs.

The United Kingdom has contributed two new people, Ed Pearce, a Visiting Fellow to Dr. Sher's group and Guest Researcher Lindsey Panton to work with the Malaria group. Staff Fellow Francis Klotz has also joined the Malaria Section, as has a guest worker Isabella Quakyi, who collaborates with the New York University group. Our three new Medical Staff Fellows have varied assignments, Beth Ungar is working on cryptosporidiosis with Dr. Nash, Tom Wellems is with the Malaria Section, and Douglas Ward has inaugurated a new arrangement to work with LCI for clinical credit and do his research with LPD for his certification in infectious diseases. Dr. Rachel Galun of Israel is with our Malaria Section as a Visiting Scientist and Dr. Alberto Makael of Venezuela will work for a short period on a special project with Jim Dvorak.

Drs. Ottesen and Nutman spent several weeks in Madras, India, along with Dr. Ron Crystal of the NHLBI initiating pulmonary lavage studies on tropical pulmonary eosinophilia patients. Dr. Carter is spending the year in Australia and in Papua, New Guinea on malaria field research. Dr. Howard initiated some work in the Gambia utilizing *P. falciparum* malaria strains from patients, and Dr. Steve Aley tested some new techniques in Israel developed by Dr. Cabantchik's lab. Dr. Neva explored possible collaboration on visceral leishmaniasis with a new ICMR lab in Patna, India, and then went on to Ethiopia where he studied cases of diffuse cutaneous leishmaniasis at the Armauer Hanson Research Institute.

Laboratory of Parasitic Diseases

National Institute of Allergy and Infectious Diseases

Summary - October 1, 1983 - September 30, 1984

HONORS AND AWARDS

The most notable recognition for any member of LPD during the past year was the news that Dr. Louis Miller will share the prestigious Paul Ehrlich Award with Drs. Ruth Nussenzweig of New York University and Ernest Beuding of Johns Hopkins. This prize, named after the famous German microbiologist-pharmacologist, is given at intervals on behalf of the West German government for accomplishments in the field of infectious diseases.

Several members of the LPD staff serve on various Committees of the W.H.O. Tropical Diseases Research Program. Dr. Ottesen continues as Chairman of the Scientific Working Group on Filariasis and Drs. Miller, Dwyer, Sher and Dvorak are on Steering Committees for Malaria, leishmaniasis and schistosomiasis, and Chagas' disease, respectively.

The following Staff members were asked to serve as organizing chairman for workshops or Symposia for the International Congress of Tropical Medicine and Malaria to be held in Calgary, Canada in September: Drs. Diamond, Gwadz, Nash, Neva and Ottesen.

Dr. Louis Diamond is President of the American Society of Protozoologists this year.

Most of the tenured Staff serve on one or more Editorial Boards of scientific journals.

Laboratory of Parasitic Diseases

National Institute of Allergy and Infectious Diseases

Summary - October 1, 1983 - September 30, 1984

RESEARCH ACCOMPLISHMENTS

MOLECULAR BIOLOGY,
AND IMMUNOCHEMISTRY
OF MALARIA

Sporozoite gene cloned by new method:

The approach generally used to isolate and characterize genes requires substantial amounts of the material (whether a parasite, hormone, etc.) to prepare genomic DNA directly or complementary DNA (cDNA). Then a series of steps ensue

in which fragments of the gene are transferred to plasmids, which are then introduced into bacteria or yeast, via various maneuvers to effect expression of the gene--with hope that sufficient amounts of the product of interest will be produced. A new method was used which allows extracting genes directly from malarial parasite DNA with a nuclease from the mung bean. The intact genes are then ligated into an expression vector, phage lambda gt-11, and the genomic DNA library is screened with immune sera for the phage plaques containing gene products of interest (McCutchan and Dame). The technique has several important advantages--relatively small amounts of starting genomic DNA is required, and exacting conditions do not seem to be required for gene expression in lambda gt-11. Using this technique the gene for the circumsporozoite (CS) protein of P. falciparum has been cloned and its nucleotide sequence determined. Repeating nucleotide sequences of the CS protein were demonstrated, and synthetic peptides of the repeat sequence inhibited binding of monoclonal antibodies to the CS protein. (Dame, McCutchan, Miller, and WRAIR collaborators). Thus, this recombinant DNA method may have useful application in identification and characterization of immunogenic microbial proteins.

Evolution by DNA structure - One human malaria more closely related to parasites from mice and birds than to those from monkeys: Species of malarial parasites (genus Plasmodium) occur throughout the animal kingdom, with many morphologic and biologic similarities between human and monkey malarias. However, the most important human species, P. falciparum, has a single component CsCl density band of DNA containing 18 per cent dG.dC, as do rodent and avian parasites. In contrast, P. vivax, a different human parasite, has multiple density bands with the major band having a dG.dC content of 30 percent, a characteristic of monkey malarias (McCutchan, Dame and Miller). Monoclonal antibodies to sporozoite antigens of P. cynomolgi, a monkey malaria, from different geographic regions do not cross-react, in contrast to geographic uniformity of sporozoites of other species of malaria (Gwadz). Analysis of the DNA of different geographic strains of P. cynomolgi also shows considerable differences, indicative of incipient speciation (Gwadz and McCutchan).

Biologic significance of surface membrane antigens still not clear: The story of surface membrane constituents on malaria-infected red cells has become more complicated than ever. The adherence of late developmental stage P. falciparum to endothelium is of great pathogenetic significance and the adherence occurs at the site of knobs (K) on the red cell surface. Several

different histidine rich proteins (His RP) have been associated with knobs; one of these, of M_r 90,000, is definitely under the surface membrane and attached to the red cell cytoskeleton. But a HisRP component of M_r 70,000 is also present, in K- as well as in K+ parasites. The two HisRPs have different solubility properties. However, a larger, surface exposed and variable size protein, M_r 260,000 to 290,000, which is exquisitely sensitive to trypsin and can be labeled with ¹²⁵I is also associated with cytoadherence. Its relationship to the His RP is still not clear (Howard, Miller and collaborators). There appears to be considerable antigenic diversity in human P. falciparum isolates in the Gambia as evidenced by reactivity with monoclonal antibody (Mab) probes. The antigens reactive with the Mabs show considerable diversity in size. While this, in itself, does not constitute evidence for antigenic variation of P. falciparum, it is hoped that the issue can be investigated further (Howard and collaborators in the Gambia).

Antigens on sexual stage malaria parasites: By western-blot analysis and radio-immunoassay it was found that only one antigenic site is the target of two monoclonal antibodies (Mabs) that block fertilization of P. falciparum gametes (Kumar and Carter). The significance of these sexual stage antigens is enhanced by the finding that the gametocytes of 20 strains of P. falciparum from New Guinea all react with the same two Mabs (Carter and collaborators in New Guinea). Attempts are being made to clone out the genes that produce these antigens. Rabbit polyclonal antibody, produced vs. P. vivax gametes from patients in Sri Lanka, have completely blocked infectivity of this parasite to mosquitoes in membrane feeding experiments (Carter and K. Mendis in Sri Lanka).

Attempt to immunize with merozoite antigen: The M_r 140,000 merozoite surface protein of P. knowlesi was column and gel purified and incorporated into liposomes to immunize rabbits and monkeys. Invasion blocking antibodies developed in one rabbit and 2 of 4 monkeys. The two monkeys with blocking antibodies controlled their infections, but the parasite which later developed was no longer blocked by immune serum and shows evidence of being a mutant or variant (Miller).

OTHER INTRACELLULAR

PROTOZOA

Leishmanial parasite membranes: The proton-pumping and ATPase activity of leishmanial surface membranes, which may be critical for intracellular parasite survival, was shown to be disrupted by chlorimipramine. This or similar drugs that uncouple oxidative phosphorylation, or inhibit proton ATPases may be worthy of further examination as antiparasitic agents (Zilberstein and Dwyer). C-DNA and genomic libraries have been constructed from several leishmanial species and stages to identify and isolate functionally relevant genes (Sheppard and Dwyer). A large number of surface membrane constituents, consisting of proteins, glycoproteins and even glycolipids, have been identified using a variety of labeling and biochemical techniques. In addition, several types of enzyme activities characteristic of leishmania can be demonstrated in infected tissues (Dwyer, Gottlieb, Wassef). Although the potential clinical utility of such assays has been noted, they have yet to be realized.

Factors controlling leishmanial infection and disease: While no simple set of comprehensive principles are yet available to explain the variable

spectrum of leishmanial infection and events responsible for disease, increasingly certain characteristics of the different parasites and factors relating to host response are being identified. Further details characterizing the infective stationary growth phase of promastigotes were found to apply to organisms from culture as well as in the sandfly vector, and could be related to lectin binding and greater resistance to oxygen intermediates (Sacks and Perkins). Yet, why is it virtually impossible to establish infection of macrophages with certain species of leishmania? Some of these same species produce no lesions in susceptible BALB/c mice (Neva). Susceptibility to killing by activated macrophages might be one answer, since this correlates to some degree with production of lesions in mice, or to growth in macrophages. However, the mechanism for macrophage killing must be something other than the O₂ burst story, since some leishmanial species are readily killed in a lymphokine-activated macrophage cell line (IC-21) that lacks an O₂ burst (Scott). Temperature was previously found to affect survival of certain leishmanial species directly, but reduced temperature was also found to impair macrophage killing of certain species of leishmania causing cutaneous disease (Scott). Long-term persistence of organisms in the footpad of BALB/c was found to be characteristic of several isolates of L. tropica minor, most of which were associated with cases of chronic relapsing disease (Neva). Addition of exogenous IL-2 failed to restore lymphocyte blastogenic responses in Ethiopian patients with DCL; they also failed to respond to local heat treatment (Neva and collaborators).

Progress on pathogenesis riddle of Chagas' disease: In spite of the tremendous amount of work on T. cruzi and Chagas' disease, the ultimate explanation for how the chronic heart and intestinal pathology of this disorder is caused remains unclear. There is increasing evidence that chronic Chagas' disease is caused not only by certain strains, but by specific clones of T. cruzi. Further long-term experiments with mice and rats relate cardiac pathology, including EKG changes observed with special equipment, to certain parasite clones, while other clones cause no significant pathology (Postan, Dvorak and collaborators). The concept of cell-mediated auto-immune pathogenesis was not supported by spleen cell transfer experiments (Postan and Dvorak). Preferential intracellular localization of T. cruzi was found to type I skeletal muscle fibers, the same type which constitute heart muscle (Teixeria and Dvorak). The accumulating mass of data on T. cruzi clones concerning characteristics such as cell volume, DNA content, growth rate, drug sensitivity, etc. has led to multivariant analysis to sort out the variables. The associations found by this approach may be helpful in understanding the biology of this parasite (Dvorak and collaborators).

Surface properties of T. cruzi and biologic function: T. cruzi¹²⁵I epimastigotes are rapidly lysed by the alternate complement pathway. By labelling of parasites, followed by treatment with C8-deficient human serum and recovery of the C3-antigen complex from lysed parasites, the acceptor molecule for C3 on the parasite surface was identified. The C3 acceptor turned out to be the same 72,000 MW glycoprotein previously studied with monoclonal Abs (Kirchhoff, Sher and Joiner of LCI). An epimastigote cDNA library in the expression vector lambda gt-11 has been constructed, and reactive clones with antibodies to the 72k protein have been found so it may be possible to isolate and sequence the gene for this protein (Lanar and Sher). Surface labeling of

clones by ¹²⁵I has started to characterize surface antigens (McDaniel). The anti-trypanosomal factor (ATF) extracted from Pseudomonas sp. was found to contain palmitoleic and oleic acids, probably components of a peptidolipid that hopefully can be further characterized (Mercado and collaborators in NIADDK).

HELMINTH INFECTIONS

Dissecting the immune response to human helminthic infections: In the process of studying antibody response to parasite antigens in vitro, a line of helper human T-cells was developed which is capable of specific blastogenic response to filarial antigen. The cell line can also mediate induction of parasite-specific antibody of the IgG, IgM and IgE isotype, but presence of HLA-DR histocompatible presenting cells are required for these responses (Nutman, Volkman of LIR and Ottesen). Additional evidence suggesting that IgE blocking antibody is IgG₄, includes the fact that the IgG₄ subclass is antigen specific, and that both the IgG₄ and IgE recognize the same antigens by immunoblotting. In addition, filarial specific IgG₄ is present in highest levels in those groups of patients with greatest blocking antibody activity (Hussain and Ottesen). While microfilarial destruction associated with eosinophil and mast cell degranulation, complement activation and a rise in serum levels of eosinophil granule protein are now well documented in the early (24-84 hrs) Mazzotti reaction, the cause of later (4 day) events after treatment of onchocerciasis with DEC is not so clear (Francis, Ottesen, LCI and Ghana collaborators). Helper T-cells (OKT 4+) were shown to be necessary for in vitro granuloma formation to S. mansoni eggs, and tests of cells at different times after infection indicated that even the in vitro response was modulated with time (Ottesen and Nash in collaboration with Doughty at Texas A & M). Early results with an immediate hypersensitivity skin-test using larval antigens for diagnosis of strongyloidiasis are encouraging (Neva).

Immunochemical studies of human parasites: The heterogeneity of Giardia organisms from man and animals, as initially revealed by DNA analysis, will require more work before epidemiologic significance can be sorted out. Although some 13 isolates have been studied by restriction patterns, surface labeling, reactivity with antisera and analysis of excretion-secretion products, no final conclusions as to human vs. animal strains can yet be drawn (Nash, Diamond and Keister). The use of Western blot techniques to examine antibody responses to schistosome antigens is being explored (Nash). A 200 kd glycoprotein filarial antigen, detected by immunoblotting, has been demonstrated either free or in complexes in the circulation of filarial patients (Paranjape, Hussain and Ottesen). Another approach has been to detect filarial antigen after dissociation from immune complexes (Lunde and Ottesen).

Experimental genetics of pathology in schistosomiasis: The lack of association between granuloma size around eggs with degree of liver fibrosis previously found with S. mansoni was confirmed in S. japonicum system in mice. However, anti-IgM treatment of mice, which results in B cell depletion, resulted in persistence of large granulomas as well as increased liver fibrosis with both parasites - presumably due to inhibition of suppressor T cell maturation. Additional evidence favoring T cell dependence of granuloma formation as well as liver fibrosis, was found in nude mice infected with S. japonicum (Cheever).

Experimental genetics of immunity in schistosomiasis: By classical cross-breeding and back cross experiments in mice the resistance defect to

vaccination with irradiated cercariae of S. mansoni reported last year was shown to be controlled by a single gene (Sher and Oliveira). In this system using irradiated cercariae, the lung was shown to be the major site of immunologic attack and parasite elimination (Sher and von Lichtenberg of Harvard). Preliminary results indicate that a soluble antigen can be identified in schistosome worm extract that is equal to irradiated cercariae in capacity to immunize vs. challenge infection (Sher in collaboration with James of G.W. Univ.).

Molecular biology of schistosomes: An adult worm cDNA library has been constructed in the expression vector, lambda gt-11, and a series of clones identified which express fusion proteins cross-reactive with schistosomulae (Lanar, Pearce and Sher). A genomic alteration was detected in the DNA of schistosomes in which hycanthone resistance had been induced (McCutchan, Sher and Beuding of Johns Hopkins). Since the drug is a frame shift mutagen, the genomic difference is probably not due to selection of a already present form, and should therefore be a useful marker for study of resistance induced by other drugs and in genetic crosses of parasites.

LUMINAL PROTOZOA

As with music, the range of work with amebae ranges from classical to modern: Some may wonder why such mundane efforts continue in research on amebae as improved methods for their growth. But the history of progress in amebiasis is closely linked to such efforts because these organisms initially require bacterial associates to grow, whereas "clean" or axenic organisms are needed for biochemical and immunologic studies. Therefore, improvements and modifications in growing amebae permit investigation of a wider range of their properties. Colonial growth of E. histolytica between double layers of agar, with plating efficiency of 35 to 100 percent, should ease the study of their indigenous lytic viruses. Preliminary results indicate that agar plating may permit direct axenization from ameba-bacterial cultures (Diamond and Aust-Kettis). More detailed study of variable conditions under which concanavalin A binds to amebae still do not associate this property with virulence (Aust-Kettis and Perez). Axenically grown E. histolytica has provided material to characterize DNA fragments of the parasite genome and to search for tubulin genes in this organism (Collaboration of Wirth and French of Harvard with Diamond).

Energy metabolism of Giardia: In contrast to E. histolytica both coenzyme A and ADP were found necessary in Giardia for aerobic oxidation of pyruvate (Weinbach). Calmodulin the regulatory protein for calcium metabolism, has been purified from Giardia and found to be similar to bovine brain calmodulin in molecular weight (20,000), enzyme activity, and transport kinetics (Munoz and Weinbach).

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| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00094-25 LPD | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Luminal Protozoa: Nutrition, Differentiation, Virulence, DNA Hybridization | | | | | | | | | | | | | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: L. S. Diamond</td> <td style="width: 33%;">Section Head</td> <td style="width: 33%;">LPD, NIAID</td> </tr> <tr> <td>Others: A. Aust-Kettis</td> <td>Guest Researcher</td> <td>LPD, NIAID</td> </tr> <tr> <td>I. B. Perez</td> <td>Guest Worker</td> <td>LPD, NIAID</td> </tr> <tr> <td>D. B. Kesiter</td> <td>Biologist</td> <td>LPD, NIAID</td> </tr> </table> | | | PI: L. S. Diamond | Section Head | LPD, NIAID | Others: A. Aust-Kettis | Guest Researcher | LPD, NIAID | I. B. Perez | Guest Worker | LPD, NIAID | D. B. Kesiter | Biologist | LPD, NIAID |
| PI: L. S. Diamond | Section Head | LPD, NIAID | | | | | | | | | | | | |
| Others: A. Aust-Kettis | Guest Researcher | LPD, NIAID | | | | | | | | | | | | |
| I. B. Perez | Guest Worker | LPD, NIAID | | | | | | | | | | | | |
| D. B. Kesiter | Biologist | LPD, NIAID | | | | | | | | | | | | |
| COOPERATING UNITS (if any) Dept. Tropical Public Health, Harvard University, (D. Wirth and C. French) | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Parasitic Diseases | | | | | | | | | | | | | | |
| SECTION Growth and Differentiation | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | |
| TOTAL MAN-YEARS 4.8 | PROFESSIONAL 2.2 | OTHER 2.6 | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td><input type="checkbox"/> (a) Human subjects</td> <td><input type="checkbox"/> (b) Human tissues</td> <td><input checked="" type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td><input type="checkbox"/> (a2) Interviews</td> <td></td> <td></td> </tr> </table> | | | <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input checked="" type="checkbox"/> (c) Neither | <input type="checkbox"/> (a1) Minors | | | <input type="checkbox"/> (a2) Interviews | | | | | |
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input checked="" type="checkbox"/> (c) Neither | | | | | | | | | | | | |
| <input type="checkbox"/> (a1) Minors | | | | | | | | | | | | | | |
| <input type="checkbox"/> (a2) Interviews | | | | | | | | | | | | | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Plating of <u>Entamoeba histolytica</u> and <u>E. invadens</u> as cloned colonies or as lawns of amebae was achieved by use of a double layer agar technique and incubation of the plates in a microaerophilic atmosphere for the former species and an anaerobic atmosphere for the latter. Cloning efficiencies were high. Strains exhibited distinct colony morphology, a factor possibly useful in classification. A lipoprotein-cholesterol fraction of bovine serum replaced whole serum in the axenic cultivation of four <u>Entamoeba</u>, <u>E. histolytica</u>, <u>E. histolytica</u>-like Laredo amebae, <u>E. invadens</u> and <u>E. moshvoskii</u>. Concanavalin A induced agglutination and the number of binding sites for the lectin on the surface of <u>E. histolytica</u> showed no correlation with the virulence of the strains examined. Moreover, SDS-PAGE, Southern blot studies showed no major qualitative differences in the membrane components of eight strains of the amebae. A putative restriction map for the ribosomal cistron of <u>E. histolytica</u> has been derived by probing enzyme digested amebic DNA with the cloned ribosomal cistron of <u>Plasmodium lophurae</u>. Ribosomal genes of <u>E. histolytica</u> appear to be highly repeated and in tandem array. The ribosomal cistron is at least 20,000 base pairs in length, and there appears to be large amounts of transcribed and non-transcribed spacer regions, excellent candidates for diagnostic probes. Extensive trials to find tubulin hybridizing sequences in <u>E. histolytica</u> restriction cut DNA using alpha- and beta-tubulin probes of <u>Leishmania enrietti</u> have been unsuccessful and suggest the absence of tubulin genes in this ameba. Suckling mice were used to advantage to obtain axenic cultures of several new strains of <u>Giardia intestinalis</u> from humans. Mice infected with cysts derived from feces served as sources of trophozoites which are more amenable to cultivation than the cyst stage. </p> | | | | | | | | | | | | | | |

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| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00097-26 LPD | | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Physiological and Cytochemical Pathology of Parasitic Diseases | | | | | | | | | | | | | | | | | | | | | | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: T. I. Mercado</td> <td style="width: 33%;">Research Physiologist</td> <td style="width: 33%;">LPD, NIAID</td> </tr> <tr> <td>Others: V. Ferrans</td> <td>Chief, Ultrastructure Sec.</td> <td>PB, NHLBI</td> </tr> <tr> <td>H.D. Hochstein</td> <td>Chief, Biological Testing and Reference Standards Branch</td> <td>DCA, BOB</td> </tr> <tr> <td>K.G. Rice</td> <td>Research Chemist</td> <td>LC, NIADDK</td> </tr> <tr> <td>M. Rafferty</td> <td>Guest Worker</td> <td>LC, NIADDK</td> </tr> <tr> <td>T. Burke</td> <td>Senior Staff Fellow</td> <td>LC, NIADDK</td> </tr> <tr> <td>L. Amende</td> <td>Senior Staff Fellow</td> <td>LCDB, NIADDK</td> </tr> </table> | | | PI: T. I. Mercado | Research Physiologist | LPD, NIAID | Others: V. Ferrans | Chief, Ultrastructure Sec. | PB, NHLBI | H.D. Hochstein | Chief, Biological Testing and Reference Standards Branch | DCA, BOB | K.G. Rice | Research Chemist | LC, NIADDK | M. Rafferty | Guest Worker | LC, NIADDK | T. Burke | Senior Staff Fellow | LC, NIADDK | L. Amende | Senior Staff Fellow | LCDB, NIADDK |
| PI: T. I. Mercado | Research Physiologist | LPD, NIAID | | | | | | | | | | | | | | | | | | | | | |
| Others: V. Ferrans | Chief, Ultrastructure Sec. | PB, NHLBI | | | | | | | | | | | | | | | | | | | | | |
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| M. Rafferty | Guest Worker | LC, NIADDK | | | | | | | | | | | | | | | | | | | | | |
| T. Burke | Senior Staff Fellow | LC, NIADDK | | | | | | | | | | | | | | | | | | | | | |
| L. Amende | Senior Staff Fellow | LCDB, NIADDK | | | | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any). Fermentation Unit, Frederick Cancer Research Facility, NCI, Frederick, MD. | | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Parasitic Diseases | | | | | | | | | | | | | | | | | | | | | | | |
| SECTION Physiology and Biochemistry | | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | | | | | | |
| TOTAL MAN-YEARS: 1.0 | PROFESSIONAL: 1.0 | OTHER: 0.0 | | | | | | | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) Studies on the chemical characterization of an anti-trypanosomal factor (ATF) from the bacterial species, <u>Pseudomonas fluorescens</u> , disclosed that the active fraction of the antibiotic substance isolated from plated cultures contained palmitoleic and oleic acids. These unsaturated fatty acids produced instantaneous lysis of <u>Trypanosoma equiperdum</u> and <u>T. cruzi</u> . Additional observations suggested that structurally the ATF is a peptidolipid. The elucidation of the peptide component of the molecule will be the subject of further studies on the chemical structure of this compound. The aim of the project is to synthesize the lytic factor and proceed with biological testing in experimental infections with <u>Trypanosoma cruzi</u> . | | | | | | | | | | | | | | | | | | | | | | | |

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AT 00098-28 LPD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Biochemical Mechanisms of Energy Metabolism in Mammalian and Parasitic Organisms

PRINCIPAL INVESTIGATOR (List either professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: E. C. Weinbach Section Head LPD, NIAID

| | | | |
|---------|----------------|---------------------------|-------------|
| Others: | L. Munoz | Visiting Fellow | LPD, NIAID |
| | J. L. Costa | Staff Physician | CNB, NIMH |
| | P. S. Ebert | Research Chemist | LMO, NCI |
| | C. E. Claggett | Bio. Lab. Tech. (Chemist) | LPD, NIAID |
| | S. C. Wieder | Bio. Lab. Tech. (Chemist) | LPD, NIAID |
| | L. Levenbook | Research Chemist | LPB, NIADDK |

COOPERATING UNITS (if any)

Department of Biochemistry, University of Stockholm (B.D. Nelson, T. Hundal)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Physiology and Biochemistry

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

PROFESSIONAL

OTHER

4

2

2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies of aerobic metabolism in *Giardia lamblia* have disclosed a novel enzyme system which catalyzes the oxidation of pyruvate in the presence of coenzyme A, and couples the oxidation to the synthesis of ATP. Calmodulin, isolated from disrupted trophozoites of axenized *G. lamblia* has been purified by hydrophobic and ion exchange chromatography to apparent homogeneity (MW=20,000). The protozoan calmodulin also has been purified in large quantities by Fast Protein Liquid Chromatography and shown to retain its biological activity (activation of phosphodiesterase). Partial characterization of the purified protein has been accomplished. In an attempt to assess the physiological role of calmodulin in *G. lamblia*. A study of calcium transport was initiated. Intact trophozoites rapidly accumulate Ca^{2+} in the presence of glucose and P_i . The uptake is inhibited by known inhibitors of calmodulin, indicative of its regulatory role in the transport of this vital cation. Continued studies of mammalian mitochondria have shown that the tricyclic antidepressant drugs imipramine and chlorimipramine have multiple, adverse effects on energy metabolism. The latter drug also has cidal actions on the enteric protozoa, and on *Leishmania donovani* (see Dwyer's report #00162-08 LPD). Initial studies of energy metabolism in human platelets showed that the tricyclic drugs also inhibit the respiratory burst elicited by thrombin. Further studies are planned to evaluate the physiological significance of these interesting findings.

Apart from the intrinsic interest of investigating a process vital to all living cells, this project has demonstrated how fundamental studies in one area (mammalian bioenergetics) can lead to unexpected, and possibly, applied results in another (parasite metabolism, and cidal action of antidepressant drugs).

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|---|----------------------|---|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00099-14 LPD |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biophysical Parasitology | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: J. A. Dvorak Res. Microbiologist LPD, NIAID | | |
| COOPERATING UNITS (if any) BEIB, DRS, (C. Gibson, W. Schuette); Harvard School of Public Health, (R. Hoff); WHO, (M.L. Teixeira) | | |
| LAB/BRANCH Laboratory of Parasitic Diseases | | |
| SECTION Physiology and Biochemistry | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MAN-YEARS: 2.0 | PROFESSIONAL: 1.0 | OTHER: 1.0 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.) <p> This project is concerned with studies of the genetic diversity of <u>Trypanosoma cruzi</u> and the implications of this diversity in the presentation and course of Chagas' disease. Major emphasis during the course of the year has centered on four topics: 1) A continuation of the multivariant analysis of <u>T. cruzi</u> clones; 2) An analysis of the surface proteins of <u>T. cruzi</u> clones; 3) The development and testing of an ultra-high resolution flow cytometer; 4) A histochemical characterization of skeletal muscle fibers of mice infected with <u>T. cruzi</u>. </p> <p> The multivariant analysis has progressed to the point where it can be demonstrated that several variables such as total DNA/cell, growth rate, allopurinol riboside sensitivity or resistance, etc. are highly associated. The virulence of a <u>T. cruzi</u> clone is related to this association. Consequently, these results support the concept that parasite genetic heterogeneity plays an important role in the presentation and course of Chagas' disease. </p> <p> A high resolution flow cytometer incorporating many features not found in commercially available instruments has become fully operational after a year of intensive development and testing. The unique capabilities of this instrument should prove useful in the analysis of numerous biochemical and physiological parameters of medically important protozoa. </p> <p> The surface components of 4 <u>T. cruzi</u> clones have been studied extensively. Major and minor proteins are shared by all 4 clones. However, several clone-specific proteins were also detected. </p> <p> The leg muscles of mice infected with <u>T. cruzi</u> were typed histochemically. Although markedly more type II fibers were present, nearly 5-fold more type I fibers were infected than type II. This is the first demonstration of a common denominator to explain the <u>in vivo</u> distribution of <u>T. cruzi</u> in muscle fibers. </p> | | |

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00102-10 LPD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pathogenesis of Disease Caused by Infection with Intracellular Parasites

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: F. A. Neva Chief LPD, NIAID

Others: D. Sacks Senior Staff Fellow LPD, NIAID

P. Scott Staff Fellow LPD, NIAID

COOPERATING UNITS (if any)

University of Arizona, Tucson, AZ, (E. A. Petersen); Harvard University Medical School, Boston, MA, (F. von Lichtenberg); Armauer Hansen Research Institute (AHRI), Addis Ababa, Ethiopia, (G. Bjune).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Cell Biology and Immunology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Leishmanial isolates from patients continue to provide a rich source of material for experimental studies. The susceptible BALB/c mouse is proving especially useful in classification of leishmanial species. Four isolates of L. tropica minor, for example, exhibit long persistence at the site of inoculation in BALB/c footpads without producing a lesion. The sequential development of lesions with different leishmanial parasites causing cutaneous disease is being studied in the ears of BALB/c mice. A technique that permits accurate localization to the injection site is utilized. Earliest histologic changes are seen within 24-48 hours after inoculation.

Five patients with the unusual anergic form of diffuse cutaneous leishmaniasis (DCL) were studied in Ethiopia. Local heat therapy failed to eliminate parasites from skin lesions of three of the patients who were tested. Lymphocytes from the DCL patients were unresponsive to leishmanial antigens in vitro, even after addition of exogenous IL-2.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00103-17 LPD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunological Studies on Toxoplasmosis and Other Parasitic Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. N. Lunde Research Zoologist LPD, NIAID

Others: E. A. Ottesen Senior Investigator LPD, NIAID
 L. Jacobs Scientist Emeritus NIAID
 A. Cheever Assistant Chief LPD, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host-Parasite Relations Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

1.1

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Sera from Clinical Center patients with AIDS continued to be monitored for antibodies to Toxoplasma gondii and Entamoeba histolytica and were found to be similar to those you would expect to find in any group of non-AIDS patients. Toxoplasma has been isolated from brain tissue of 5 AIDS patients at autopsy. A method has been developed to detect filarial antigen in circulating immune complexes of patients with filariasis. The method is not influenced by rheumatoid, anti-nuclear and other non-specific factors sometimes contained in serum. The method will not detect circulating unbound antigen so that the antigen detected is only antigen which was dissociated from the polyethylene glycol precipitated immune complexes. Antigen was found in complexes from 9 of 32 sera from filariasis patients in India and in 5 of 12 sera tested from filariasis patients in the Cook Islands. These sera were previously found to have circulating immune complexes by both the Clq-ELISA and ¹²⁵I-Clq binding assay. In addition sera from a patient with no demonstrable filarial antigen complexes before treatment with diethylcarbamazine had antigen detected in immune complexes after initiation of treatment.

The method provides a method of evaluating immune complexes in these patients as to whether or not the complexes are related to filariasis or are due to some other factors not related to filariasis.

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|--|-----------------------|---|-----------------|-----------------------|------------|----------------------|---------------------|------------|-----------|--------------|------------|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00108-13 LPD | | | | | | | | | |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Studies on the Biology and Immunogenicity of Malaria Sporozoites | | | | | | | | | | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: R. W. Gwadz</td> <td style="width: 33%;">Research Entomologist</td> <td style="width: 33%;">LPD, NIAID</td> </tr> <tr> <td>Others: T. McCutchan</td> <td>Senior Staff Fellow</td> <td>LPD, NIAID</td> </tr> <tr> <td>I. Quakyi</td> <td>Guest Worker</td> <td>LPD, NIAID</td> </tr> </table> | | | PI: R. W. Gwadz | Research Entomologist | LPD, NIAID | Others: T. McCutchan | Senior Staff Fellow | LPD, NIAID | I. Quakyi | Guest Worker | LPD, NIAID |
| PI: R. W. Gwadz | Research Entomologist | LPD, NIAID | | | | | | | | | |
| Others: T. McCutchan | Senior Staff Fellow | LPD, NIAID | | | | | | | | | |
| I. Quakyi | Guest Worker | LPD, NIAID | | | | | | | | | |
| COOPERATING UNITS (if any) New York University, School of Medicine, (R. Nussenzweig, A. Cochrane, E. Enea) | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Parasitic Diseases | | | | | | | | | | | |
| SECTION Malaria | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | |
| TOTAL MAN-YEARS 2.6 | PROFESSIONAL 1.0 | OTHER 1.6 | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | | | | | | | | | | |
| SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided) <p> <u>Circumsporozoite (CS) proteins</u> have been shown to act as the <u>protective antigens</u> capable of inducing <u>sporozoite-specific immunity</u> in several species of malaria. <u>Monoclonal antibodies</u> raised against <u>surface antigens</u> derived from specific geographic isolates of <u>Plasmodium falciparum</u>, <u>P. vivax</u> and <u>P. berghei</u> react with other isolates of the same species. However, monoclonal antibodies raised against sporozoites of the simian malaria, <u>P. cynomolgi</u>, from one area do not react with sporozoites of other geographic isolates of that species. Analysis of the organization of the DNA within and surrounding certain genes of these <u>P. cynomolgi</u> isolates show significant differences indicative of at least incipient speciation. <u>P. vivax</u> appears to be a member of the <u>P. cynomolgi</u> complex of species. </p> | | | | | | | | | | | |

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00161-07 LPD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunochemistry of Parasitic Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: T. E. Nash Senior Scientist LPD, NIAID

Others: L. S. Diamond Section Head LPD, NIAID
M. N. Lunde Research Zoologist LPD, NIAID
D. B. Keister Biologist LPD, NIAID
A. W. Cheever Assistant Chief LPD, NIAID
B. Ungar Medical Staff Fellow LPD, NIAID
J. A. Dvorak Research Microbiologist LPD, NIAID

COOPERATING UNITS (if any)

Animal Parasitology Institute, Beltsville, Md. (R. Fayer), Michigan State University (J. Bennett), University of Khartoum (M. Homeida).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host Parasite Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

.75

.75

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies are being performed on three parasitic parasites: Schistosomes, Giardia, and Cryptosporidium. Antibody responses in acute and chronic schistosomiasis have been compared using Western blots. Major differences between these two groups were noted and antibody responses to some antigens are predominately seen in acute or early infections. The surface antigens of Giardia and human antibody responses to Giardia antigens were compared using a variety of isolates. Studies indicate major differences in the surface antigens of Giardia isolates and the humoral responses of man are sometimes specific to the homologous isolate. Studies of Cryptosporidium have just begun with the aim of developing a measure of antibody responses and assays for specific parasite antigen in stool.

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| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00162-08 LPD |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biochemical Cytology of Host-Parasite Interactions in Parasitic Protozoa | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) PI: D. M. D. Dwyer Supervisory Microbiologist LPD, NIAID | | |
| Others: H. W. Sheppard Staff Fellow LPD, NIAID M. K. Wassef Res. Chemist/Guest Invest. USFDA/LPD, NIAID D. Zilberstein Guest Investigator LPD, NIAID T. B. Fioretti Chemist LPD, NIAID M. K. Kurtz Biologist LPD, NIAID | | |
| COOPERATING UNITS (if any) Dept. of Immunology & Infectious Diseases, The Johns Hopkins University, (M. Gottlieb); Bur. Vet. Med., USFDA, DHHS | | |
| LAB/BRANCH Laboratory of Parasitic Diseases | | |
| SECTION Cell Biology & Immunology | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MAN-YEARS 5.0 | PROFESSIONAL 3.5 | OTHER: 1.5 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The cell biology, biochemistry and immunology of <u>Leishmania</u> and <u>Trypanosoma</u> are investigated as models of intra- and extracellular parasitism, respectively. As all interactions between host and parasite occur, at least temporally, at the level of the parasite surface membrane, emphasis is placed on: 1) integrated structural, biochemical and antigenic characterization of the intact and isolated parasite surface membrane (SM) and parasite released products and 2) defining the mechanisms by which parasites survive and circumvent host-defense systems. To those ends, various studies are employed involving: Subcellular fractionation, radiolabeling, electrophoresis and chromatography assays, immuno-binding and labeling assays and <u>in vitro</u> culture.</p> <p>Using such techniques, the major SM components/antigens of <u>Leishmania</u> sp. were identified. The major SM antigens to which leishmaniasis patients make IgG responses were delineated. Total lipid composition of <u>Leishmania</u> SM was determined and 3 SM phospholipases characterized. The SM origin and characterization of <u>Leishmania</u>-released antigens was demonstrated. Antibodies, C-DNA and genomic libraries were made for isolation of SM antigens and their genes. The kinetics and mechanisms of <u>Leishmania</u> SM sugar and amino acid transport were delineated. Both a SM-proton pump and a specific [H⁺]-ATPase were identified. The former providing the electro-chemical potential for SM transport in this organism. These results underscore the relevance of the parasite surface membrane and the need for its biochemical and immunochemical characterization.</p> <p>The goals of this project are to provide fundamental bases for understanding the mechanisms of parasite survival.</p> | | |

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| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00197-05 LPD |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immune Recognition in Filariasis and Other Helminth Infections | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: R. Hussain Senior Staff Fellow LPD, NIAID Others: E. A. Ottesen Senior Investigator LPD, NIAID H. A. Francis Medical Staff Fellow LPD, NIAID T. E. Nutman Medical Staff Fellow LPD, NIAID R. Paranjape Visiting Fellow LPD, NIAID | | |
| COOPERATING UNITS (if any) Johns Hopkins Medical Center, Baltimore, Md., University of Berne, Switzerland | | |
| LAB/BRANCH Laboratory of Parasitic Diseases | | |
| SECTION Host Parasite Relations | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MAN-YEARS: 1.75 | PROFESSIONAL: 1.0 | OTHER: 0.75 |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) The major aim of this project is to characterize immunoglobulin responses in helminth infections (primarily filariasis and schistosomiasis) with emphasis on IgE production, regulation and modulation. Sensitive radioimmunoassays have been developed and utilized for quantitating IgG and IgE antibodies. Qualitative characterization in terms of what antigens are being recognized in various clinical forms of the disease is being carried out to understand immune recognition and its implication in the pathogenesis and/or defense of the disease. These studies would in addition provide information about antigens with better specificity in immunodiagnosis or epidemiologic studies. Finally, <u>in vitro</u> production and regulation of IgE synthesis is also under investigation to better understand the control mechanisms of IgE production. | | |

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00208-04 LPD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Isolation and Characterization of Plasmodial Genes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: T. McCutchan Senior Staff Fellow LPD, NIAID

Others: J. Dame Staff Fellow LPD, NIAID
J. Mullins Biologist LPD, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

3.0

PROFESSIONAL

2.0

OTHER

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have developed a method for culling intact genes out of Plasmodium DNA with mung bean nuclease. The intact genes are then ligated in the expression vector lambda gtn in order to make a recombinant gene bank containing genes from all stages of the parasites life cycle. The gene banks can then be screened with immune sera for bacteriophage containing particular genes of interest. Using this approach we have isolated several genes which may produce antigenic peptides that would be useful in the preparation of a vaccine for malaria including the circumsporozoite gene of P. falciparum.

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|--|---|---|------------------|---|------------|---------------|---------------|------------|--------------|--------------|------------|--------------|--------------|------------|-------------|------------------|------------|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00240-03 LPD | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Culture, Physiology and Antigenic Analysis of Sexual Stages of Malaria Parasites | | | | | | | | | | | | | | | | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: R. Carter Visiting Scientist LPD, NIAID | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">Others: N. Kumar</td> <td style="width: 33%;">Burroughs Wellcome Senior Research Fellow</td> <td style="width: 33%;">LPD, NIAID</td> </tr> <tr> <td>L. A. McNicol</td> <td>W.H.O. Fellow</td> <td>LPD, NIAID</td> </tr> <tr> <td>T. McCutchan</td> <td>Staff Fellow</td> <td>LPD, NIAID</td> </tr> <tr> <td>L. H. Miller</td> <td>Section Head</td> <td>LPD, NIAID</td> </tr> <tr> <td>R. W. Gwadz</td> <td>Senior Scientist</td> <td>LPD, NIAID</td> </tr> </table> | | | Others: N. Kumar | Burroughs Wellcome Senior Research Fellow | LPD, NIAID | L. A. McNicol | W.H.O. Fellow | LPD, NIAID | T. McCutchan | Staff Fellow | LPD, NIAID | L. H. Miller | Section Head | LPD, NIAID | R. W. Gwadz | Senior Scientist | LPD, NIAID |
| Others: N. Kumar | Burroughs Wellcome Senior Research Fellow | LPD, NIAID | | | | | | | | | | | | | | | |
| L. A. McNicol | W.H.O. Fellow | LPD, NIAID | | | | | | | | | | | | | | | |
| T. McCutchan | Staff Fellow | LPD, NIAID | | | | | | | | | | | | | | | |
| L. H. Miller | Section Head | LPD, NIAID | | | | | | | | | | | | | | | |
| R. W. Gwadz | Senior Scientist | LPD, NIAID | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) See Next Page | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Parasitic Diseases | | | | | | | | | | | | | | | | | |
| SECTION Malaria | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | |
| TOTAL MAN-YEARS: 5.6 | PROFESSIONAL: 3.3 | OTHER: 2.3 | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Further characterization of the target antigens of two transmission blocking monoclonal antibodies (Mabs) in <u>Plasmodium falciparum</u> indicate that of the 3 proteins immunoprecipitated by these Mabs (255, 59 and 53 kilodaltons (kDa)), only the 255 kDa protein carries the epitopes with which the Mabs react, the 59 and 53 kDa proteins being apparently co-precipitated. Immunoradiometric assays confirm that the two Mabs, IIC5-B10 and IA3-B8, react with distinct epitopes on the target protein; the data is consistent with each epitope being represented once only on the target molecule. Following protease treatment and analysis of peptide fragments of the 255 kDa protein no evidence was obtained of structural differences in this protein between several isolates of <u>P. falciparum</u>, including one isolate previously shown to be variant at one epitope on this protein.</p> <p>The two main surface proteins, 26 and 28 kDa, synthesized and expressed on zygotes of <u>Plasmodium gallinaceum</u> during transformation to ookinetes, of which the 26 kDa is the target of a transmission blocking Mab, were previously shown to be both glycosylated and to contain covalently bound fatty acid. However, based on differential precipitation by different Mabs and polyclonal immune sera, and different patterns of protease digestion fragments, the two proteins do not otherwise appear to be structurally related.</p> <p>A genomic expression library from <u>P. falciparum</u> is being screened for clones of <u>E. coli</u> containing recombinant DNA (in the lambda gt 11 vector) coding for gamete surface antigens using polyclonal and monoclonal antibodies against the sexual stages of <u>P. falciparum</u>.</p> <p>Transmission blocking antibodies have been successfully raised against a second species of human malaria, <u>Plasmodium vivax</u>, by immunization of rabbits with gametes of this parasite derived from natural human infections.</p> | | | | | | | | | | | | | | | | | |

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|---|---------------------|---|------------------|--------------|------------|--|--|--|------------------|--------------|------------|-----------|-------------------|------------|-----------|-----------|------------|---------------|--------------------|--------|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00241-03 LPD | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Identification of Receptors for Merozoite Invasion of Erythrocytes | | | | | | | | | | | | | | | | | | | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: L. H. Miller</td> <td style="width: 33%;">Section Head</td> <td style="width: 33%;">LPD, NIAID</td> </tr> <tr> <td colspan="3"> </td> </tr> <tr> <td>Others: F. Klotz</td> <td>Staff Fellow</td> <td>LPD, NIAID</td> </tr> <tr> <td>R. Howard</td> <td>Expert Consultant</td> <td>LPD, NIAID</td> </tr> <tr> <td>D. Hudson</td> <td>Biologist</td> <td>LPD, NIAID</td> </tr> <tr> <td>M. McGinninis</td> <td>Research Biologist</td> <td>BB, CC</td> </tr> </table> | | | PI: L. H. Miller | Section Head | LPD, NIAID | | | | Others: F. Klotz | Staff Fellow | LPD, NIAID | R. Howard | Expert Consultant | LPD, NIAID | D. Hudson | Biologist | LPD, NIAID | M. McGinninis | Research Biologist | BB, CC |
| PI: L. H. Miller | Section Head | LPD, NIAID | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | |
| Others: F. Klotz | Staff Fellow | LPD, NIAID | | | | | | | | | | | | | | | | | | |
| R. Howard | Expert Consultant | LPD, NIAID | | | | | | | | | | | | | | | | | | |
| D. Hudson | Biologist | LPD, NIAID | | | | | | | | | | | | | | | | | | |
| M. McGinninis | Research Biologist | BB, CC | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) Walter Reed Army Institute of Research, Washington, D.C., (T. Hadley and G. Lyons); Hazelton Laboratories, Vienna, Va. (J. Rener); Case Western Reserve University, Cleveland, OH (M. Aikawa); CDC, Atlanta, Ga. (G. Campbell) | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Parasitic Diseases | | | | | | | | | | | | | | | | | | | | |
| SECTION Malaria | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | | | |
| TOTAL MAN-YEARS 2.5 | PROFESSIONAL 1.5 | OTHER 1.0 | | | | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <p> The <u>merozoite</u> interacts in a <u>receptor</u> specific manner with the <u>erythrocyte</u> surface and is the stage against which immunity may work to block <u>invasion</u>. Thus, merozoite surface components are of interest for their role in erythrocyte recognition and as <u>antigens</u> for induction of <u>protective immunity</u>. We are now studying the processing of these molecules during the ultimate stages of parasite development, their role in reception and as immunogens for induction of protective immunity. </p> <p> The receptors on the erythrocyte (Duffy blood group determinant for <u>Plasmodium knowlesi</u> and <u>P. vivax</u> and glycophorin for <u>P. falciparum</u>) will be used to affinity purify the parasite antigens. </p> | | | | | | | | | | | | | | | | | | | | |

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00242-03 LPD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biological and Biochemical Studies of Antigens on Malaria-infected Red Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|-----|-----------|-------------------|------------|
| PI: | R. Howard | Expert Consultant | LPD, NIAID |
|-----|-----------|-------------------|------------|

| | | | |
|---------|--------------|--------------------|------------|
| Others: | L. H. Miller | Section Head | LPD, NIAID |
| | J. H. Leech | Clinical Associate | LPD, NIAID |
| | J. Sherwood | Clinical Associate | LPD, NIAID |
| | S. B. Alely | Staff Fellow | LPD, NIAID |
| | W. Daniel | Research Associate | LPD, NIAID |
| | V. Kao | Technician | LPD, NIAID |

COOPERATING UNITS (if any)

See next page

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

5.5

PROFESSIONAL:

4

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
 ☐ (b) Human tissues
 ☒ (c) Neither
- ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

We are studying the structure and function of malarial proteins inserted into the membrane of erythrocytes infected with mature asexual malaria parasites. Three malarial proteins have been identified at this site by our work to date.

Two *P. falciparum* proteins are associated with knob protrusions of the erythrocyte membrane. Knobs mediate attachment of infected erythrocytes to endothelium, thereby preventing passage of infected cells through the spleen. A *P. falciparum* protein on the surface of the erythrocyte membrane has properties consistent with a role in cytoadherence: this protein displays exquisite trypsin sensitivity, as does cytoadherence to endothelium, and, strain-specific sera which block or reverse cytoadherence of the homologous strain specifically immunoprecipitate this antigen from the homologous strain. This molecule exhibits size diversity between strains (M_r 260,000-290,000) in addition to antigenic diversity. The second erythrocyte membrane-associated *P. falciparum* protein is of unusually high histidine content (histidine-rich protein, HisRP). This protein is localized underneath the erythrocyte membrane at knobs. Another HisRP of unknown function is soluble in neutral detergents, in contrast to the knob-associated HisRP.

The *Plasmodium knowlesi* variant antigen on the surface of infected rhesus monkey erythrocytes is the third erythrocyte surface antigen. This parasite protein (M_r 180,000-230,000) varies antigenically in cloned organisms as a parasite immune evasion mechanism. Differential extraction experiments with detergents and protein show that this molecule is very tightly associated with the membrane, probably with both the lipid membrane and underlying cytoskeleton.

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| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00244-03 LPD |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Developmental Adaptations of <u>Trypanosoma cruzi</u> to the Vertebrate Immune System | | |
| PRINCIPAL INVESTIGATOR (List either professional personnel below, the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: F. A. Sher Section Head LPD, NIAID Others: L. V. Kirchhoff Medical Staff Fellow LPD, NIAID J. Dvorak Senior Investigator LPD, NIAID D. Lanar Guest Worker LPD, NIAID | | |
| COOPERATING UNITS (if any) Wellcome Research Laboratories, Kent, England (D. Snary); Laboratory of Clinical Investigation, NIAID (K. Joiner) | | |
| LAB/BRANCH Laboratory of Parasitic Diseases | | |
| SECTION Immunology and Cell Biology Section | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MAN-YEARS 1.4 | PROFESSIONAL 1.2 | OTHER 0.2 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> In this project, we have been studying developmental adaptations of <u>Trypanosoma cruzi</u> to the vertebrate host and, in particular, surface membrane changes occurring during the morphogenesis of epimastigotes (vector stage) to trypomastigotes (vertebrate stage). During the past year we focussed most of our effort in studying interactions of the complement system with these parasite stages and, in particular we investigated the epimastigote acceptor site for C3 during activation of the alternative pathway (AP). Epimastigotes were surface labeled with ¹²⁵I by the iodogen method and reacted with fresh human serum. The parasites were then lysed in detergent and the C3-parasite membrane complex purified by affinity chromatography on anti-C3 Sepharose. Electrophoretic and immunochemical analysis revealed that the C3 had bound selectively to one <u>T. cruzi</u> surface component - a 72,000 MW_R glycoprotein. Minimal C3 deposition was observed when metacyclic trypomastigotes (derived from <u>in vitro</u> culture) were tested in the same reaction. These results provide the first demonstration of a C3 acceptor molecule on a parasite. </p> <p> In related projects, an epimastigote cDNA library was constructed in the expression vector lambda gt-11 in preparation for identification of the gene encoding the 72 kd glycoprotein. A <u>T. cruzi</u> insect vector, <u>Dipetalogaster maximus</u>, was propagated in preparation for studies on bug derived metacyclic trypomastigotes. </p> | | |

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00246-02 LPD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Studies of the Genome and Surface of Schistosoma mansoni

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: F. A. Sher Section Head LPD, NIAID

| | | | |
|---------|--------------|---------------------|------------|
| Others: | D. Lanar | Guest Worker | LPD, NIAID |
| | R. Oliveira | Guest Worker | LPD, NIAID |
| | T. McCutchan | Senior Staff Fellow | LPD, NIAID |
| | E. J. Pearce | Fogarty Fellow | LPD, NIAID |

COOPERATING UNITS (if any)

Biomedical Research Institute, Rockville, MD; Johns Hopkins Univ, (E. Bueding)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Immunology and Cell Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.7

PROFESSIONAL:

1.5

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been resumed after a one year inactive period because of change of personnel. The long range goals of the project are to understand the genomic organization of schistosomes, identify target antigens on the worms and clone genes encoding these antigens for the purpose of vaccine production. During the past year progress was achieved in the following areas: (1) monoclonal antibodies were produced from mice immunized with an irradiated vaccine and an anti-schistosomulum surface monoclonal partially characterized: (2) An adult worm cDNA library was constructed in the expression vector lambda gt-11 and a series of clones identified which express fusion proteins cross-reactive with schistosomula: (3) a genomic alteration was detected in the DNA of schistosomes as a consequence of the induction of resistance to the chemotherapeutic drug hycanthone.

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| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00248-03 LPD | | | |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | | | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Physiology and Genetics of Anopheles Mosquitoes | | | | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) PI: R. W. Gwadz Research Entomologist LPD, NIAID | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 33%; vertical-align: top;"> Others: R. Galun I. Quakyi V. Barbiero L. Koontz K. Vernick </td> <td style="width: 33%; vertical-align: top;"> Visiting Scientist Guest Worker Guest Worker Biologist Bio. Lab. Tech. </td> <td style="width: 33%; vertical-align: top;"> LPD, NIAID LPD, NIAID LPD, NIAID LPD, NIAID LPD, NIAID </td> </tr> </table> | | | Others: R. Galun I. Quakyi V. Barbiero L. Koontz K. Vernick | Visiting Scientist Guest Worker Guest Worker Biologist Bio. Lab. Tech. | LPD, NIAID LPD, NIAID LPD, NIAID LPD, NIAID LPD, NIAID |
| Others: R. Galun I. Quakyi V. Barbiero L. Koontz K. Vernick | Visiting Scientist Guest Worker Guest Worker Biologist Bio. Lab. Tech. | LPD, NIAID LPD, NIAID LPD, NIAID LPD, NIAID LPD, NIAID | | | |
| COOPERATING UNITS (if any) Red Cross Blood Center, Bethesda, MD, (R. Williams & T. Takahashi) | | | | | |
| LAB/BRANCH Laboratory of Parasitic Diseases | | | | | |
| SECTION Malaria | | | | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | | | | |
| TOTAL MAN-YEARS 4.6 | PROFESSIONAL 1.4 | OTHER 3.2 | | | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | | | | |
| SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <p> Research has focused on determining the <u>genetic</u> and <u>physiological</u> basis of <u>susceptibility</u> or <u>refractoriness</u> to <u>malaria</u> in <u>anopheline mosquitoes</u>. Studies have emphasized defining refractoriness in selected strains of the <u>African malaria vector, Anopheles gambiae</u>. In one strain, refractoriness is manifested by the death and <u>encapsulation</u> of early malarial oocysts on the mosquito gut. Backcross studies indicate that refractoriness is probably controlled by two unlinked loci, one of which has a major effect on refractoriness. The second locus is closely linked to an isoenzyme locus. The precise mode of inheritance of refractoriness is being determined. </p> | | | | | |

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00251-03 LPD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunologic Studies on Schistosomiasis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: F. A. Sher Section Head LPD, NIAID

Others: E. J. Pearce Fogarty Fellow LPD, NIAID
 A. Cheever Assistant Chief LPD, NIAID
 A. J. G. Simpson Fogarty Fellow LPD, NIAID
 D. L. Sacks Senior Staff Fellow LPD, NIAID

COOPERATING UNITS (if any)

Biomedical Research Institute, Rockville, MD, (F.A. Lewis); George Washington University, (S.L. James); Harvard University (F. von Lichtenberg); Immunology Branch, NCI, (A. Singer).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Immunology and Cell Biology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

3.2

PROFESSIONAL:

2.5

OTHER:

0.7

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Studies were carried out this year on the genetics of irradiated vaccine induced immunity, the histopathology of the irradiated vaccine model, the explanation for incomplete killing of challenge infections in vaccinated mice, and the tissue origin of schistosome MHC antigens. In addition, in collaboration with Dr. S. James the purification and characterization of a vaccine immunogen was initiated.

The resistance defect of P/N mice to vaccination with irradiated cercariae was shown to be controlled by a single gene and to be unlinked to another single gene defect controlling IgM anti-schistosomulum antibody synthesis. In a related study another IgM deficiency controlled by the Xid gene was shown not to effect vaccine induced immunity. In a separate project the failure of vaccine induced immunity to entirely eliminate infections was shown not to be due to the existence of immunologically resistant parasite sub-populations. Finally, in a histopathological study, the lung was shown to be a major site of challenge attrition in vaccinated mice.

The tissue origin of schistosome MHC antigens was examined using worms recovered from radiation chimera mice. Class II (IA) antigens were shown to be hemopoietically derived whereas Class I (K region) antigens were demonstrated to originate from a non-hemopoietic source.

Mice vaccinated with frozen and thawed schistosomula plus BCG were shown by Dr. James to be immune to challenge infection. In collaboration with Dr. James we showed that a soluble fraction of the larvae mediates the same effect and that the vaccine immunogens are heat and pronase sensitive.

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| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00253-03 LPD |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders) Studies of the Immunologic Responses to Filarial Infections | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: E. A. Ottesen Senior Investigator LPD, NIAID | | |
| Others: SEE NEXT PAGE | | |
| COOPERATING UNITS (if any) Tuberculosis Research Center, Madras, India, (S.P. Tripathy); Medical College of Madras, India (K.V. Thiruvengadam); Johns Hopkins Medical Center, (R.G. Hamilton); University of Berne, Switzerland (F. Skvaril) | | |
| LAB/BRANCH Laboratory of Parasitic Diseases | | |
| SECTION Host Parasite Relations | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MAN-YEARS 3.8 | PROFESSIONAL 2.6 | OTHER 1.2 |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The purpose of this project is to define the humoral and cellular immune responses that relate to immunopathology, protective immunity and immunodiagnosis of patients with filariasis.</p> <p>To account for the marked quantitative differences in IgE production by patients with different clinical manifestations of filariasis, lymphocyte regulatory mechanisms have been studied. Blood mononuclear cells from helminth infected patients spontaneously produce abundant IgE <u>in vitro</u> in contrast to those from normals or atopic individuals; the mechanistic reasons for this are being examined. A filarial antigen specific T-cell line and clones have been developed and utilized to show that antigen presentation is genetically controlled (DR-restricted) and that factors mediating B-cell activation to produce IgE can be identical and distinguished from those leading to production of other immunoglobulins. Use of sensitive qualitative techniques (immunoblotting) to analyse IgE antibodies in infected patients has revealed differences in allergen recognition unappreciated in prior studies.</p> <p>The presence of IgG blocking antibodies that inhibit immediate hypersensitivity responses to parasite antigen correlates with clinical findings in infected patients and, thus, may have pathogenetic significance. Analysis of IgG subclasses has shown extreme elevations of IgG4 antibody in the response to infection. Furthermore, there is profound qualitative similarity between IgE and IgG4 responses to parasite antigens; the possibility that IgG4 is the blocking antibody is being investigated.</p> <p>Identification of filarial antigens in the circulation of patients has been successful using a 2-site immunoradiometric assay and immunoblotting. A single, specific heat-stable glycoprotein of 200 kd m.w. can be identified in all microfilaremic patients with <u>W. bancrofti</u> filariasis and some without microfilaremia. Isolation of the antigen and production of monoclonal antibodies to it are underway.</p> | | |

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|--|-----------------------|---|-------------------|---------------------|------------|--|--|--|--------------------|----------------------|------------|------------|---------------------|------------|-------------|-------|------------|------------|-----------------------|------------|--------------|----------------------|---------|----------------|-------|---------|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00254-03 LPD | | | | | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies of the Host Response in Onchocerciasis | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: E. A. Ottesen</td> <td style="width: 33%;">Senior Investigator</td> <td style="width: 33%;">LPD, NIAID</td> </tr> <tr><td colspan="3"> </td></tr> <tr> <td>Others: H. Francis</td> <td>Medical Staff Fellow</td> <td>LPD, NIAID</td> </tr> <tr> <td>R. Hussain</td> <td>Senior Staff Fellow</td> <td>LPD, NIAID</td> </tr> <tr> <td>M. M. Frank</td> <td>Chief</td> <td>LCI, NIAID</td> </tr> <tr> <td>M. Kaliner</td> <td>Head, Allergy Section</td> <td>LCI, NIAID</td> </tr> <tr> <td>T. J. Lawley</td> <td>Medical Investigator</td> <td>DB, NCI</td> </tr> <tr> <td>R. Nussenblatt</td> <td>Chief</td> <td>CB, NEI</td> </tr> </table> | | | PI: E. A. Ottesen | Senior Investigator | LPD, NIAID | | | | Others: H. Francis | Medical Staff Fellow | LPD, NIAID | R. Hussain | Senior Staff Fellow | LPD, NIAID | M. M. Frank | Chief | LCI, NIAID | M. Kaliner | Head, Allergy Section | LCI, NIAID | T. J. Lawley | Medical Investigator | DB, NCI | R. Nussenblatt | Chief | CB, NEI |
| PI: E. A. Ottesen | Senior Investigator | LPD, NIAID | | | | | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Others: H. Francis | Medical Staff Fellow | LPD, NIAID | | | | | | | | | | | | | | | | | | | | | | | | |
| R. Hussain | Senior Staff Fellow | LPD, NIAID | | | | | | | | | | | | | | | | | | | | | | | | |
| M. M. Frank | Chief | LCI, NIAID | | | | | | | | | | | | | | | | | | | | | | | | |
| M. Kaliner | Head, Allergy Section | LCI, NIAID | | | | | | | | | | | | | | | | | | | | | | | | |
| T. J. Lawley | Medical Investigator | DB, NCI | | | | | | | | | | | | | | | | | | | | | | | | |
| R. Nussenblatt | Chief | CB, NEI | | | | | | | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) Onchocerciasis Chemotherapeutic Res. Ctr., Tamale, Ghana, (K. Awadzi, and D. Badu); Dept. of Med., Mayo Clinic, Rochester, MN, (C.J. Gleich and S.K. Ackerman); Spec. Prog. for Trop. Dis. Res., WHO, Geneva, Switzerland | | | | | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Parasitic Diseases | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SECTION Host Parasite Relations | | | | | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TOTAL MAN-YEARS: 1.0 | PROFESSIONAL: 1.0 | OTHER: 0.0 | | | | | | | | | | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The goal of this project is to study the host response to onchocercal infection in order to understand the pathogenesis of clinical disease, the immune mechanisms important in the persistence of the parasite within the host and in protective immunity, and to develop improved immunodiagnostic techniques.</p> <p>The initial phase has involved detailed clinical and laboratory assessment of the severe side effects (Mazzotti reaction) that accompany treatment of the infection and limit the potential of mass chemotherapy for onchocerciasis. Twenty-four patients were treated at a research center in Ghana and subjected to intensive immunologic and clinical evaluation during their Mazzotti reactions. Hypotension, fever, adenitis and puritis all were correlated with infection intensity after detailed statistical analyses were carried out. Most prominent of the immunological changes was a dramatic fall of serum complement levels within 2 hours of initiating treatment and evidence for activation of immediate hypersensitivity immune mechanisms, the latter including evidence of eosinophil and mast cell degranulation both morphologic around microfilariae being killed in the skin and biochemical with rises in serum eosinophil granule protein concentrations. Specific IgE antibodies (assessed by histamine release and radioimmunoassay) and extremely high levels of total IgE were demonstrated pre-treatment and likely were important in mast cell degranulation after antigen was released from dying parasites. Thus, immediate hypersensitivity immune mechanisms, whether triggered by complement-derived anaphylotoxin, specific IgE antibody or eosinophil-derived proteins appear as important determinants of the Mazzotti reaction in man.</p> <p>Also in progress is a study of the immunopathology of onchocercal eye lesions using ocular tissue and fluids removed from patients with onchocerciasis at the time of cataract surgery carried out in Ghana.</p> | | | | | | | | | | | | | | | | | | | | | | | | | | |

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|---|---|---|--|---|--------------------------------------|--------------------------------------|--|--|---|---------------------|------------|---------------|----------------------|------------|--------------|----------------------|------------|------------|---------------------|------------|---------------|-------|-----------|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER ZO1 AI 00255-03 LPD | | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders) Studies of the Immunologic Responses to Non-Filarial Helminth Infections | | | | | | | | | | | | | | | | | | | | | | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: E. A. Ottesen</td> <td style="width: 33%;">Senior Investigator</td> <td style="width: 33%;">LPD, NIAID</td> </tr> <tr> <td colspan="3"> </td> </tr> <tr> <td>Others: R. Hussain</td> <td>Senior Staff Fellow</td> <td>LPD, NIAID</td> </tr> <tr> <td>C. J. Maxwell</td> <td>Medical Staff Fellow</td> <td>LPD, NIAID</td> </tr> <tr> <td>T. B. Nutman</td> <td>Medical Staff Fellow</td> <td>LPD, NIAID</td> </tr> <tr> <td>T. E. Nash</td> <td>Senior Investigator</td> <td>LPD, NIAID</td> </tr> <tr> <td>R. G. Crystal</td> <td>Chief</td> <td>PB, NHLBI</td> </tr> </table> | | | PI: E. A. Ottesen | Senior Investigator | LPD, NIAID | | | | Others: R. Hussain | Senior Staff Fellow | LPD, NIAID | C. J. Maxwell | Medical Staff Fellow | LPD, NIAID | T. B. Nutman | Medical Staff Fellow | LPD, NIAID | T. E. Nash | Senior Investigator | LPD, NIAID | R. G. Crystal | Chief | PB, NHLBI |
| PI: E. A. Ottesen | Senior Investigator | LPD, NIAID | | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | |
| Others: R. Hussain | Senior Staff Fellow | LPD, NIAID | | | | | | | | | | | | | | | | | | | | | |
| C. J. Maxwell | Medical Staff Fellow | LPD, NIAID | | | | | | | | | | | | | | | | | | | | | |
| T. B. Nutman | Medical Staff Fellow | LPD, NIAID | | | | | | | | | | | | | | | | | | | | | |
| T. E. Nash | Senior Investigator | LPD, NIAID | | | | | | | | | | | | | | | | | | | | | |
| R. G. Crystal | Chief | PB, NHLBI | | | | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) Texas A & M University (B. Doughty); Tulane University (B. Cline, S. Katz, D. Little); San Juan Laboratories, CDC (R. A. Hiatt). | | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Parasitic Diseases | | | | | | | | | | | | | | | | | | | | | | | |
| SECTION Host Parasite Relations | | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | | | | | | |
| TOTAL MAN-YEARS 1.25 | PROFESSIONAL 1.25 | OTHER 0 | | | | | | | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td><input checked="" type="checkbox"/> (a) Human subjects</td> <td><input checked="" type="checkbox"/> (b) Human tissues</td> <td><input type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td colspan="2"></td> </tr> <tr> <td><input checked="" type="checkbox"/> (a2) Interviews</td> <td colspan="2"></td> </tr> </table> | | | <input checked="" type="checkbox"/> (a) Human subjects | <input checked="" type="checkbox"/> (b) Human tissues | <input type="checkbox"/> (c) Neither | <input type="checkbox"/> (a1) Minors | | | <input checked="" type="checkbox"/> (a2) Interviews | | | | | | | | | | | | | | |
| <input checked="" type="checkbox"/> (a) Human subjects | <input checked="" type="checkbox"/> (b) Human tissues | <input type="checkbox"/> (c) Neither | | | | | | | | | | | | | | | | | | | | | |
| <input type="checkbox"/> (a1) Minors | | | | | | | | | | | | | | | | | | | | | | | |
| <input checked="" type="checkbox"/> (a2) Interviews | | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.) <p> In Schistosomiasis mechanisms involved in the progressive modulation of the granulomatous response to schistosome eggs were studied <u>in vitro</u> by using peripheral blood mononuclear cells from patients with early or chronic infection. Development of the granulomas required the presence of OKT4+ (helper) lymphocytes; OKT8+ (suppressor) cells inhibited granuloma formation. Patients with early infection had greater peri-oval granulomatous responses than did those with older infections. IgE responses of acutely infected patients, however, were less than those with chronic infection but the differences appeared mostly quantitative as qualitative analyses by immunoblotting techniques showed that essentially all allergens that were going to be recognized by a given individual were recognized very early. </p> <p> Experimental hookworm (<u>Necator americanus</u>) infections in man have been established with a goal toward defining progressive changes in immunologic responses (especially IgE) to helminth infection. </p> | | | | | | | | | | | | | | | | | | | | | | | |

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| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00256-03 LPD |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Differentiation of <u>Leishmania</u> Promastigotes | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: D. L. Sacks Senior Staff Fellow LPD, NIAID Others: A. Sher Section Head LPD, NIAID | | |
| COOPERATING UNITS (if any) Department of Entomology, Walter Reed Army Institute of Research (P. V. Perkins) | | |
| LAB/BRANCH Laboratory of Parasitic Diseases | | |
| SECTION Immunology and Cell Biology Section | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MAN-YEARS: 1.5 | PROFESSIONAL | OTHER: |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Sequential development of <u>Leishmania</u> promastigotes from a noninfective to an infective stage has been observed for parasites growing both within culture and the sandfly vector. The generation of infective stage promastigotes is growth cycle related and restricted to non-dividing organisms. Thus parasites taken from the logarithmic phase of growth in culture and the sandfly midgut are relatively avirulent for a susceptible vertebrate host (BALB/c mice), whereas those taken from stationary cultures or later midgut infections are highly virulent. The increase in virulence is related to the parasite's ability to resist the normal microbicidal activities of host macrophages, and not in their ability to gain entrance into these cells. A comparisons of the susceptibility of log and stationary promastigotes to oxygen-dependent antimicrobial mechanisms revealed that the later were 2-3 times more resistant to the lethal effects of the oxygen intermediate, hydrogen peroxide. This could explain the ability of infective stage promastigotes to survive the respiratory burst triggered by the rapid uptake of these organisms. Infective and non-infective stage promastigotes can be separated on the basis of their differential binding to the lectin, peanut agglutinin. Comparison of these relatively homogeneous stage specific populations will permit further identification of those specific antigenic, biochemical, and physiological changes which accompany transition of these parasites from invertebrate to vertebrate environments and which determine the successful parasitism of <u>Leishmania</u> within the vertebrate host.</p> | | |

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|--|--------------------------|---|----------------|-------|------------|----------------------|-------------------|-------------|------------|-----------------|--|------------|--------------------------|----------------|-----------|-----------------|------------|--|----------------------|------------|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00257-03 LPD | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Immunology of Strongyloidiasis | | | | | | | | | | | | | | | | | | | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: F. A. Neva</td> <td style="width: 33%;">Chief</td> <td style="width: 33%;">LPD, NIAID</td> </tr> <tr> <td>Others: W. T. London</td> <td>Chief, Section on</td> <td>IRP, NINCDS</td> </tr> <tr> <td>E. Ottesen</td> <td>Exp. Parasitol.</td> <td></td> </tr> <tr> <td>K. Barrett</td> <td>Head, Clinical Parasitol</td> <td>LCI/LPD, NIAID</td> </tr> <tr> <td>T. Nutman</td> <td>Visiting Fellow</td> <td>LCI, NIAID</td> </tr> <tr> <td></td> <td>Medical Staff Fellow</td> <td>LPD, NIAID</td> </tr> </table> | | | PI: F. A. Neva | Chief | LPD, NIAID | Others: W. T. London | Chief, Section on | IRP, NINCDS | E. Ottesen | Exp. Parasitol. | | K. Barrett | Head, Clinical Parasitol | LCI/LPD, NIAID | T. Nutman | Visiting Fellow | LCI, NIAID | | Medical Staff Fellow | LPD, NIAID |
| PI: F. A. Neva | Chief | LPD, NIAID | | | | | | | | | | | | | | | | | | |
| Others: W. T. London | Chief, Section on | IRP, NINCDS | | | | | | | | | | | | | | | | | | |
| E. Ottesen | Exp. Parasitol. | | | | | | | | | | | | | | | | | | | |
| K. Barrett | Head, Clinical Parasitol | LCI/LPD, NIAID | | | | | | | | | | | | | | | | | | |
| T. Nutman | Visiting Fellow | LCI, NIAID | | | | | | | | | | | | | | | | | | |
| | Medical Staff Fellow | LPD, NIAID | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) Meloy Laboratories, Rockville, MD, (J. Cismanec); Veteran's Administration Hosp., Wichita, Kansas (L. Pelletier) | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Parasitic Diseases | | | | | | | | | | | | | | | | | | | | |
| SECTION Cell Biology and Immunology | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | | | |
| TOTAL MAN-YEARS 0.8 | PROFESSIONAL 0.4 | OTHER 0.4 | | | | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Since excretion of <u>Strongyloides stercoralis</u> larvae in infected individuals is often scanty and irregular, diagnosis by direct fecal examination is unreliable and difficult. Therefore, we have continued to define circumstances in which serodiagnosis is useful and to explore other methods, such as the immediate hypersensitivity skin test. Further experience with the ELISA test for antibody indicates that levels decline and often become negative 1 to 2 years after successful treatment. A collaborative study of World War II U.S. prisoners of war in the Far East indicates that a substantial number of them are still infected with <u>S. stercoralis</u>. Antibody to the parasite was found in 26 percent of 180 veterans, many of whom had elevated eosinophil levels. Early results with a skin test, using antigens prepared from larvae of <u>S. stercoralis</u> and <u>S. ratti</u>, have shown positive immediate reactions in all parasitologically proven strongyloidiasis cases. Additional experience is needed to define specificity and possible cross reactions in patients infected with other parasites. </p> <p> Monoclonal antibodies have been developed in mice immunized with somatic <u>S. stercoralis</u> antigen. Of the 6 reactive clones, 3 also cross-react with filarial whole worm antigen, but the other 3 appear to have greater specificity. </p> | | | | | | | | | | | | | | | | | | | | |

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00258-03 LPD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Models for Chagas' Disease Using T. cruzi Clones and Inbred Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. Postan

WHO Fellow

LPD, NIAID

Others: J. A. Dvorak

Res. Microbiologist

LPD, NIAID

J. Bailey

Chief, Med. Application Sect.

LAS, DCRT

E. Pottala

Senior Engineer

LAS, DCRT

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Physiology and Biochemistry

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.5

PROFESSIONAL

1.0

OTHER

0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

T. cruzi clones and inbred mice are being used to develop experimental models for Chagas' disease. These studies demonstrate that a myocardiotropic clone of T. cruzi induces in C3H mice a pattern of myocarditis similar to human chagasic myocarditis. The possibility of inducing myocardial inflammation and fibrosis by spleen cell transfer from mice chronically infected with a myocardiotropic T. cruzi clone to uninfected normal mice has been investigated. In contrast with other reports, preliminary data showed that, myocarditis could not be induced by spleen cell transfer even with irradiated recipient mice. The immunization of inbred mice with low virulence T. cruzi clones does not protect against challenge with more virulent clones of the parasite. The infection of rats with two T. cruzi clones that differ in their pathogenicity for inbred mice was studied. The patterns of infection of rats with the two clones were similar to that occurring in C3H mice when infected with the same T. cruzi clones. The data indicate that the outcome of infection is greatly influenced by the genetic characteristics of the parasite stock.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00347-02 LPD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Schistosomal Hepatic Fibrosis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: A. W. Cheever Assistant Chief LPD, NIAID

| | | |
|----------------------|----------------------------|------------|
| Others: R. H. Duvall | Bio. Lab. Tech. (Micro.) | LPD, NIAID |
| T. A. Hallack, Jr. | Bio. Lab. Tech. (Gen.) | LPD, NIAID |
| J. Malley | Mathematical Statistician | LSM, DCRT |
| K. Malley | Computer Assistant Analyst | LSM, DCRT |
| A. Sher | Head, Immunology Section | LPD, NIAID |

COOPERATING UNITS (if any)

George Washington University, (S. James); Brigham & Women's Hospital, Boston, Mass., Dept. of Pathology, (F. V. Lichtenberg, J. Byram)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host-Parasite Relations Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

1.5

PROFESSIONAL

0.5

OTHER

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Hepatic fibrosis is examined in mice infected with schistosome species pathogenic for man. Mouse strains developed markedly different degrees of hepatic fibrosis following infection with S. mansoni. Among inbred strains, Nmri mice showed maximal fibrosis and C57BL/6 mice minimal fibrosis. Inheritance was multigenic with incomplete dominance. Granuloma size also differed markedly between the mouse strains and was also inherited in an incompletely dominant fashion with several genes apparently involved. In backcross generations hepatic fibrosis and granuloma size segregated independently. S. japonicum infections in different mouse strains also induced variable fibrosis and the relative rank of the mouse strains was similar to that for S. mansoni; however some mouse strains (e.g. C57BL/6) having small granulomas in S. mansoni infection had large granulomas after S. japonicum infection and granuloma size was clearly not related to fibrosis. Mice without antibody (anti-u treated) formed normal granulomas around both S. mansoni and S. japonicum eggs at 7-8 weeks after infection. T cell deficient (nude) mice formed discrete granulomas around S. japonicum eggs but the lesions were much smaller than those in heterozygotes and fibrosis was minimal. Thus, contrary to expectations, T cells are much more important than antibody in the formation of S. japonicum egg granulomas and the necrotizing features of the granulomas were not antibody dependent.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00348-02 LPD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunity in Murine Schistosomiasis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: A. W. Cheever Assistant Chief LPD, NIAID

Others: R. H. Duvall Bio. Lab. Tech (Micro) LPD, NIAID
 A. Sher Section Head LPD, NIAID
 J. Malley Mathematical Statistician LSM, DCRT
 K. Malley Computer Assistant Analyst LSM, DCRT
 P. Shade Bio. Lab. Tech. LPD, NIAID

COOPERATING UNITS (if any)

Biomedical Research Institute, Rockville, MD, (P. Stirewalt, F. Lewis, and C. Richards)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host-Parasite Relations Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

2.0

PROFESSIONAL:

0.5

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Substrains of S. mansoni selected for varying infectivity to vector snails have been tested for their ability to induce immunity in mice. Two strains derived from the same patient and designated PRT-3 and PRC-3 induced markedly different degrees of resistance to reinfection after a bisexual first infection. The F-1 cross between these strains also produced high resistance, comparable to that induced by the "immunogenic" PRT-3 strain. C57BL/KsJ mice with unisexual S. mansoni infections generally were about 40% resistant to challenge infections; however, the PRC-3 strain induced no resistance after unisexual infection. The PRT-3 and PRC-3 strains have been selected for 5 generations on the basis of their ability to induce resistance, but no apparent selection of higher or lower resistance traits has been achieved.

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|--|---|---|---|---|--------------------------------------|--------------------------------------|--|--|--|--------------|------------|--------------|---------------------|------------|---------------|-----------|------------|--------------|-------------------------|------------|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00350-02 LPD | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) DNA Analysis of Parasites | | | | | | | | | | | | | | | | | | | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: T. E. Nash</td> <td style="width: 33%;">Medical Officer</td> <td style="width: 33%;">LPD, NIAID</td> </tr> <tr> <td colspan="3" style="padding-top: 10px;">Others:</td> </tr> <tr> <td>J. Dame</td> <td>Staff Fellow</td> <td>LPD, NIAID</td> </tr> <tr> <td>T. McCutchan</td> <td>Senior Staff Fellow</td> <td>LPD, NIAID</td> </tr> <tr> <td>D. B. Keister</td> <td>Biologist</td> <td>LPD, NIAID</td> </tr> <tr> <td>J. A. Dvorak</td> <td>Research Microbiologist</td> <td>LPD, NIAID</td> </tr> </table> | | | PI: T. E. Nash | Medical Officer | LPD, NIAID | Others: | | | J. Dame | Staff Fellow | LPD, NIAID | T. McCutchan | Senior Staff Fellow | LPD, NIAID | D. B. Keister | Biologist | LPD, NIAID | J. A. Dvorak | Research Microbiologist | LPD, NIAID |
| PI: T. E. Nash | Medical Officer | LPD, NIAID | | | | | | | | | | | | | | | | | | |
| Others: | | | | | | | | | | | | | | | | | | | | |
| J. Dame | Staff Fellow | LPD, NIAID | | | | | | | | | | | | | | | | | | |
| T. McCutchan | Senior Staff Fellow | LPD, NIAID | | | | | | | | | | | | | | | | | | |
| D. B. Keister | Biologist | LPD, NIAID | | | | | | | | | | | | | | | | | | |
| J. A. Dvorak | Research Microbiologist | LPD, NIAID | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) None | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Parasitic Diseases | | | | | | | | | | | | | | | | | | | | |
| SECTION Host-Parasite Relations | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | | | |
| TOTAL MAN-YEARS 0.5 | PROFESSIONAL 0.5 | OTHER 0.0 | | | | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td><input type="checkbox"/> (a) Human subjects</td> <td><input checked="" type="checkbox"/> (b) Human tissues</td> <td><input type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td><input type="checkbox"/> (a2) Interviews</td> <td></td> <td></td> </tr> </table> | | | <input type="checkbox"/> (a) Human subjects | <input checked="" type="checkbox"/> (b) Human tissues | <input type="checkbox"/> (c) Neither | <input type="checkbox"/> (a1) Minors | | | <input type="checkbox"/> (a2) Interviews | | | | | | | | | | | |
| <input type="checkbox"/> (a) Human subjects | <input checked="" type="checkbox"/> (b) Human tissues | <input type="checkbox"/> (c) Neither | | | | | | | | | | | | | | | | | | |
| <input type="checkbox"/> (a1) Minors | | | | | | | | | | | | | | | | | | | | |
| <input type="checkbox"/> (a2) Interviews | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.) <p>Comparison of the restriction endonuclease pattern of <u>Giardia lamblia</u> isolates was expanded to include 15 isolates which originated from man and animals. A large degree of heterogeneity among the isolates was noted and this technique offers to be valuable in the analysis of isolates and in epidemiological studies.</p> | | | | | | | | | | | | | | | | | | | | |

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|---|---------------|---|------------|-------------|--------------|------------|---------|---------|--------------|------------|--|----------|--------------|------------|--|------------|-------|------------|--|-------------|-----------|------------|--|----------|-----------------------|------------|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00351-03 LPD | | | | | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Parasite and Host Factors Controlling the Pathogenesis of Leishmaniasis | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI:</td> <td style="width: 33%;">P. A. Scott</td> <td style="width: 33%;">Staff Fellow</td> <td style="width: 33%;">LPD, NIAID</td> </tr> <tr> <td>Others:</td> <td>A. Sher</td> <td>Section Head</td> <td>LPD, NIAID</td> </tr> <tr> <td></td> <td>D. Sacks</td> <td>Staff Fellow</td> <td>LPD, NIAID</td> </tr> <tr> <td></td> <td>F. A. Neva</td> <td>Chief</td> <td>LPD, NIAID</td> </tr> <tr> <td></td> <td>P. Natovitz</td> <td>Biologist</td> <td>LPD, NIAID</td> </tr> <tr> <td></td> <td>D. Dwyer</td> <td>Supvr. Microbiologist</td> <td>LPD, NIAID</td> </tr> </table> | | | PI: | P. A. Scott | Staff Fellow | LPD, NIAID | Others: | A. Sher | Section Head | LPD, NIAID | | D. Sacks | Staff Fellow | LPD, NIAID | | F. A. Neva | Chief | LPD, NIAID | | P. Natovitz | Biologist | LPD, NIAID | | D. Dwyer | Supvr. Microbiologist | LPD, NIAID |
| PI: | P. A. Scott | Staff Fellow | LPD, NIAID | | | | | | | | | | | | | | | | | | | | | | | |
| Others: | A. Sher | Section Head | LPD, NIAID | | | | | | | | | | | | | | | | | | | | | | | |
| | D. Sacks | Staff Fellow | LPD, NIAID | | | | | | | | | | | | | | | | | | | | | | | |
| | F. A. Neva | Chief | LPD, NIAID | | | | | | | | | | | | | | | | | | | | | | | |
| | P. Natovitz | Biologist | LPD, NIAID | | | | | | | | | | | | | | | | | | | | | | | |
| | D. Dwyer | Supvr. Microbiologist | LPD, NIAID | | | | | | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) Wellcome Research Laboratories, Experimental Biology Div., London, England (J. Howard); Naval Biosciences Laboratories, Oakland, CA (H. Sheppard); George Washington University, Washington, D.C. (S. James). | | | | | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Parasitic Diseases | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SECTION Immunology & Cell Biology | | | | | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: | | | | | | | | | | | | | | | | | | | | | | | | |
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| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The survival of <u>Leishmania</u> depends upon their ability to evade the microbicidal activity of macrophages. We have defined two mechanisms by which the parasite escapes macrophage mediated killing. First, certain strains are resistant to lymphokine (LK)-mediated activated macrophage killing. These parasites are located at one end of a spectrum in susceptibility to macrophage killing of strains belonging to the Genus <u>Leishmania</u>. The second manner by which parasites escape killing is by residing in the skin. We have found that at cutaneous temperatures, which range between 28°C and 33°C, macrophages display an impaired capacity to kill <u>Leishmania</u>. In order to better understand both of these phenomena, we investigated the mechanism(s) used by macrophages to kill <u>Leishmania</u> parasites. We found that the macrophage cell line, IC-21, was incapable of producing oxygen metabolites often associated with killing. Nevertheless, LK-activated IC-21 cells killed <u>Leishmania</u>, suggesting that oxygen metabolites are not required to kill <u>Leishmania</u>. We are presently studying a cytolytic factor derived from these cells that may be responsible for mediating macrophage microbicidal activity. </p> <p> In a related project, we have been attempting to define the antigens involved in the induction of protective immunity with a non-living <u>L. tropica</u> vaccine. We have identified protective parasite fractions. An analysis of the antigens recognized by protected and non-protected mice revealed that certain antigens were associated with protection. We are presently using both conventional and DNA recombinant technology to further identify these protective antigens. In addition, we are using these antigens to define the immunologic mechanisms responsible for protection in vaccine mice. </p> | | | | | | | | | | | | | | | | | | | | | | | | | | |

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| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00424-01 LPD | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Intestinal Parasites of AIDS and homosexuals | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 45%;">T. E. Nash</td> <td style="width: 40%;">Senior Scientist</td> <td style="width: 20%;">LPD, NIAID</td> </tr> <tr> <td rowspan="6">Others:</td> <td>L. S. Diamond</td> <td>Section Head</td> <td>LPD, NIAID</td> </tr> <tr> <td>M. N. Lunde</td> <td>Research Zoologist</td> <td>LPD, NIAID</td> </tr> <tr> <td>D. B. Keister</td> <td>Biologist</td> <td>LPD, NIAID</td> </tr> <tr> <td>B. Ungar</td> <td>Medical Staff Fellow</td> <td>LPD, NIAID</td> </tr> <tr> <td>J. A. Dvorak</td> <td>Research Microbiologist</td> <td>LPD, NIAID</td> </tr> <tr> <td>T. McCutchan</td> <td>Senior Staff Fellow</td> <td>LPD, NIAID</td> </tr> <tr> <td></td> <td>J. Dame</td> <td>Staff Fellow</td> <td>LPD, NIAID</td> </tr> </table> | | | PI: | T. E. Nash | Senior Scientist | LPD, NIAID | Others: | L. S. Diamond | Section Head | LPD, NIAID | M. N. Lunde | Research Zoologist | LPD, NIAID | D. B. Keister | Biologist | LPD, NIAID | B. Ungar | Medical Staff Fellow | LPD, NIAID | J. A. Dvorak | Research Microbiologist | LPD, NIAID | T. McCutchan | Senior Staff Fellow | LPD, NIAID | | J. Dame | Staff Fellow | LPD, NIAID |
| PI: | T. E. Nash | Senior Scientist | LPD, NIAID | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| | T. McCutchan | Senior Staff Fellow | LPD, NIAID | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | J. Dame | Staff Fellow | LPD, NIAID | | | | | | | | | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) Animal Parasitology Institute, Beltsville, Md. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Parasitic Diseases | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SECTION Host Parasite Section | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TOTAL MAN-YEARS: .75 | PROFESSIONAL: .75 | OTHER: | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <p>A Major goal of this laboratory is the study of the biology and host responses to <i>Giardia lamblia</i> and <i>cryptosporidium</i>. Both of these parasites are prevalent and cause disease in homosexuals and AIDS patients but also cause significant morbidity in the general population. Although <i>Giardia</i> is the most common disease causing intestinal parasite in AIDS and homosexuals, it is more readily treatable with conventional drugs than <i>Cryptosporidiosis</i>, which is not treatable with currently available drugs and therefore can be a serious and lethal infection in AIDS patients.</p> <p>Research on <i>cryptosporidium</i> has only recently begun. Several isolates have been obtained and are now maintained in vivo in suckling mice. There is preliminary evidence of differences in susceptibility to genetically deficient mice. One of the major problems has been getting enough parasite to raise antibodies, monoclonals and develop test systems. Recently, a number of calves were infected with this parasite and this has enabled us to obtain large quantities of infected stools which will, after purification of cysts, yield workable quantities of parasite.</p> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

LABORATORY OF VIRAL DISEASES
1984 Annual Report
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PHS-NIH
SUMMARY REPORT

ANNUAL REPORT OF THE LABORATORY OF VIRAL DISEASES, NIAID
October 1, 1983 to September 30, 1984

Dr. Bernard Moss
Chief, Laboratory of Viral Diseases

During the past year, the Macromolecular Biology Section of the Laboratory of Biology of Viruses was transferred to the Laboratory of Viral Diseases and Dr. Bernard Moss was named laboratory chief. Although these additions have brought new programs in areas of molecular biology and vaccine development, murine retrovirus studies remain a major research effort and the inspiration provided by Dr. Wallace P. Rowe continues to guide many of the activities of LVD. Dr. Janet Hartley was converted from Acting Head to Head of the Viral Oncology section and Drs. Hilton B. Levy and Herbert Morse continue as Heads of the Molecular Virology and Viral and Cellular Immunology Sections, respectively. Dr. Christine Kozak was approved for tenure in recognition of her substantial scientific accomplishments.

The major programs of the Viral Oncology Section and the Virology and Cellular Immunology Section have a common focus in studies of the hematopoietic cell system and aberrations of that system resulting from neoplastic or autoimmune diseases.

Research in the Viral Oncology Section continues to center on the murine C-type retroviruses or murine leukemia viruses (MuLVs), especially on studies of the natural history in laboratory and wild mice of the several classes and the association of these viruses with disease. Specific areas in which progress is reported include the role of MuLV-related genes in determining susceptibility to chemical or virus induced leukemias and lymphomas, recognition and characterization of host genes controlling MuLV infection in wild mice, identification of new MuLVs of distinctive type in wild mice, characterization of a new transforming virus of defective oncogene transduction type, and the identification of viral genetic factors determining target cell specificity in certain systems.

In studies by members of the Virology and Cellular Immunology Section, a new defective transforming virus which induces an unusual type of lymphoma has been isolated and biologically and molecularly characterized and a variety of MuLV-induced B cell neoplasms have provided a means of analyzing antigenically the stages of B cell differentiation. The latter study has led to the identification of a previously unrecognized early B cell stage, a putative progenitor of pre-B cells. In studies of genes related to autoimmune disease an apparently identical T cell population change has been found in mice carrying two distinct mutant genes.

In other areas of research, serological surveys and DNA sequence homology tests have revealed no suggestion of increased level of infection with members of the Parvovirus group in AIDS or pre-AIDS patients. Current efforts are directed toward determining the role of retroviruses in AIDS. In studies of the epidemiology and control of ectromelia virus infection of

laboratory mice a commonly used vaccine was found to be largely ineffective and primary efforts have turned to the development of an effective and safe vaccine.

The Macromolecular Biology Section has continued fundamental studies on the replication and expression of poxviruses and the development of vaccinia virus as a eukaryotic cloning and expression vector. The potential use of vaccinia virus recombinants as live vaccines against a variety of infectious diseases is currently being evaluated. For both fundamental and applied studies, emphasis has been given to analyzing the promoter regions that regulate the early and late expression of vaccinia virus genes. These sequences were shown to be structurally and functionally distinct from analogous ones recognized by eukaryotic RNA polymerases. The efficient expression of foreign genes in vaccinia virus recombinants is dependent on the use of strong vaccinia virus promoters. By properly engineering the recombinants it has been possible to obtain high levels of synthesis and correct processing of foreign proteins. A single intradermal vaccination was shown to protect experimental animals against infection with hepatitis B virus, influenza virus and herpes simplex virus. Attention also is being given to the further attenuation of vaccinia virus so that the recombinants will have minimal side effects.

The Section of Molecular Virology, in collaboration with the NCI has shown that the primate effective interferon inducer Poly ICLC enhances a variety of cell associated immune reactions more effectively than any other biologic response modifier tested to date. There have been a few partial remissions in some human malignancies, with strong control in malignant juvenile laryngo-papilloma. Results with paralytic neurologic diseases of presumed dysimmune etiology, such as multiple sclerosis and peripheral neuropathy have been encouraging, but require much more extensive testing.

Some of the highlights of the current year follow:

Genetic basis for susceptibility of wild mice to xenotropic MuLVs. Cells from certain wild mice but not laboratory mice are susceptible to infection by exogenous X-MuLVs. Susceptibility is dominant and is controlled by a single locus, Sxv, which was mapped to chromosome 1, closely linked to Bxv-1, an X-MuLV induction locus. Sxv is in the same chromosomal region as the receptor locus for MCF-MuLVs and may represent an allelic variant of this locus in wild mice. (Kozak)

Molecular biology of Fv-4 resistance gene. Fv-4 is a genetic locus found in Japanese mice responsible for resistance to exogenous ecotropic MuLVs. Southern blot analysis identified a novel provirus associated with Fv-4 related to ecotropic MuLVs. Fv-4 was molecularly cloned and nucleotide sequence analysis shows that Fv-4 contains ecotropic-MuLV related envelope sequences. This is consistent with an interference model of Fv-4 resistance in which the Fv-4 gene product binds to ecotropic cell surface receptors. Further studies suggest that the Fv-4 provirus differs from laboratory mouse viral isolates in its hybridization properties and its internal restriction map. (Ikeda, Kozak (LVD); Laigret, Theodore, Repaske, Martin, LMI).

Presence of Fv-1 and Fv-2 resistance genes in wild mice. Many wild mice lack the Fv-1 restriction of N- or B-tropic MuLVs characteristic of inbred strains. Genetic studies with 2 different feral mice, M.m. praetextus and M. spretus,

show that this phenotype is determined at or near the Fv-1 locus indicating that these wild mice carry a novel Fv-1 allele. Studies with M. spretus indicate that these mice also carry the Fv-2 resistance allele responsible for resistance to Friend complex virus induced disease. (Kozak)

Genetic mapping of endogenous retroviral loci. Genetic crosses and somatic cell hybrids have been used to map more than 10 proviral loci to different mouse chromosomes. Most recently, 2 of the 3 BALB/c mouse mammary tumor viral loci were mapped to chromosomes 12 and 16, and induction loci for xenotropic MuLVs distinct from the chromosome 1 Bxv-1 locus were identified in MA/My, NZB/B1N and in wild-derived Japanese mice. (Kozak, Hartley, Morse, Callahan)

Endogenous MuLV sequences generally lacking in wild mice. Studies on wild mouse populations indicate that all of those tested except M.m. molossinus lack proviral sequences related to laboratory mouse ecotropic MuLVs. However, MuLVs with ecotropic host range have been isolated from M. hortulanus and M.m. castaneus as well as M.m. molossinus. Southern blot hybridization data indicates that viruses of all 3 mice differ from ecotropic MuLVs of laboratory mice in hybridization properties and internal restriction maps. (Kozak)

Ecotropic and MCF MuLVs use different cell surface receptors and regulation of ecotropic and xenotropic virus induction loci. Chinese hamster/mouse somatic cell hybrids were used to demonstrate that MCF and ecotropic MuLVs use different receptor loci and that the MCF receptor gene is on mouse chromosome 1. (Kozak)

Regulation of ecotropic and xenotropic virus induction loci is different. By use of somatic cell hybrids, the time course of virus induction was studied in hybrids lacking receptor loci but which contained induction loci for ecotropic (Akv-2) or xenotropic (Bxv-1) MuLVs. Results showed that even in the absence of secondary virus spread, Akv-2 and Bxv-1 differed substantially in the time course of virus induction. (Kozak)

Genetic mapping of nongermine proviruses associated with tumorigenesis. Tumors associated with long latency viruses often contain nongermine proviruses. Since these proviruses are often integrated into common chromosomal regions in independent tumors, they are thought to play an important role in tumorigenesis. Flanking sequence probes from several of these tumor-specific integration sites were used to analyze somatic cell hybrids. Results show that one of these sites is present on chromosome 7 and 4 are on chromosome 15. (Risser, Jolicoeur, Peters, Tschlis, Kozak)

Endogenous MuLV proviral sequences in mice and murine thymic lymphomas. A study of the linkage of the light chain immunoglobulin locus, Ig1-1, on mouse chromosome 16 with the Akv-2 proviral insert in AKR/J mice was performed utilizing a recombinant DNA probe derived from mouse genomic DNA flanking the Akv-2 proviral insert. The results indicated that the two genes map within 5.9 cM of each other and supported the model of the regulatory locus being identical with the structural gene for lambda light chains. (Buckler)

Wild mouse-derived defective transforming virus may be new member of v-Ras family. A new isolate of a murine acutely transforming retrovirus, recovered

from an unusual monocytic leukemia arising in a mouse-infected with a wild mouse ecotropic virus-induced recombinant MCF virus, appears to be functionally related but biochemically distinct from the known members of the oncogene family ras. In addition to allowing extension of our understanding of the relationships between oncogenes, this virus may be useful in studying the mechanism of oncogene transduction. (Hartley, Fredrickson, Hoggan)

Defective transforming virus induces pre-B cell lymphomas. Another acute transforming murine retrovirus, recovered from a wild mouse ecotropic MuLV induced pre-B cell lymphoma, induces a high frequency of tumors similar to that from which it was isolated. Preliminary data suggest that it contains a previously undescribed oncogene. (Langdon, Morse, Holmes, Hartley)

Portion of the 3' end of Moloney and Friend MuLVs specifies type of disease induced. Oncogenicity testing tests in mice of reciprocal in vitro recombinant viruses representing the genome of each virus substituted with a 0.62 kb fragment containing the U3 region of the LTR from the other, have shown that the substituted region largely controls whether the mice develop erythro-leukemia (characteristic of Friend virus) or lymphoblastic lymphoma (characteristic of Moloney virus). This suggests a relationship of specific viral enhance sequences to target cell specificity. (Hartley; Hopkins, MIT)

Role of virus sensitivity genes Fv-1 and Rmcf in genetic control of pristane-induced plasmacytogenesis. A partial clarification of the genetic basis for the difference in plasmacytoma response between BALB/c (sensitive; Fv-1^b, Rmcf^S) and DBA/2 (resistant; Fv-1ⁿ, Rmcf^r) mouse strains was obtained in tests of BALB/c congenic for Fv-1 and/or Rmcf alleles from DBA. No resistance attributable to Rmcf^r was detected, but mice congenic for Fv-1ⁿ were partially resistant. (Hartley; Potter, NCI)

Identification of AKR genes conferring high susceptibility to thymic lymphoma. Several genes besides inherited ecotropic viruses play a role in the high susceptibility of AKR mice to thymic lymphomas. To identify such genes, crosses between AKR mice and other strains were tested for the AKR pattern of rapid onset of lymphoma after inoculation with Friend helper virus. Development of lymphoma in this system was not associated with inheritance of ecotropic virus. Mice inheriting regions of chromosome 7 (near the albino locus) and chromosome 15 (near the caracul locus) from AKR had a significantly higher incidence of lymphoma, suggesting that these chromosomal regions carry susceptibility loci from AKR. (Silver)

Establishment of a series of B cell lines from lymphomatous and normal tissues. Two sets of continuous B cell lines were established in vitro, one from MuLV, induced lymphomas and the other from the spleens of normal mice. Members of the lymphoma-derived B cell series resembled normal B cells arrested at various stages of differentiation, from putative progenitors of pre-B cells (pro-B cells) to mature B cells. Many of these lines were Ly-1⁺ and one produced auto-antibodies to bromelain-treated mouse RBC. The previously undescribed pro-B cells were Ly b-2⁺, Mac1⁺, Ly-5(B220)[±]. Cells of this phenotype were also found in normal newborn mice where they represented 20% of spleen cells and 40% of bone marrow cells. The non-transformed B cell lines established from normal spleens were IgM⁺, IgD⁺, λ⁺, Ly-1⁺. These lines exhibited enhanced proliferation after stimulation with dextran sulphate or LPS, whereas their growth was inhibited by treatment with anti-IgM antibodies. A small proportion of these cells could be induced to mature

and secrete IgM. (Morse, Davidson, Holmes)

The autoimmunity genes *lpr* and *gld* cause identical abnormalities of T lymphocytes. C3H mice homozygous for the non-allelic, autosomal recessive mutations *lpr* or *gld* develop autoimmune disease and massive lymphadenopathy resulting from the expansion of a population of T cells that uniquely expresses the B cell antigen Ly-5(B220). In addition, these mice exhibit polyclonal B cell activation and defective IL-2 production. The similarities in the phenotypic and functional abnormalities induced by *lpr* and *gld* suggest that they may be mutations that affect different enzymes in a common metabolic pathway of importance to the differentiation and function of T cells. (Davidson, Morse)

Parvoviruses not implicated in genesis of AIDS. Sera from AIDS, chronic lymphadenopathy and hemophiliac patients as well as matched controls from normal and high risk populations were surveyed for the presence of abnormal levels of parovirus antibodies using the ELISA test. Additionally a collection of DNA clones representing all major groups of known paroviruses were assembled and characterized. These were used as probes in hybridization experiments to search for related sequences in various fluids and tissues of AIDS and pre-AIDS patients. The results of both antibody and molecular studies were uniformly negative. Attention is now directed to the involvement of retroviruses in AIDS. (Hoggan, Benn)

Plasmid vectors facilitate construction of vaccinia virus recombinants. The production and selection of infectious vaccinia virus recombinants expressing foreign genes was facilitated by the development of new plasmid vectors. The insertion of a continuous coding sequence for a foreign protein at one of the unique restriction endonuclease sites juxtaposes the transcriptional start site of a vaccinia promoter and the translational start site of a foreign gene. After transfection of vaccinia virus-infected cells with such plasmids, homologous recombination occurs between the vaccinia virus sequences flanking the chimeric gene and the same sequences within the virus genome. Infectious virus recombinants expressing the prokaryotic enzyme chloramphenicol acetyltransferase were constructed to optimize the system. The expression of chloramphenicol acetyltransferase was detected within 1 hour after infection of cells with recombinant virus when early promoters were used and at 6 hours after infection when late promoters were used. (Smith, Weir and Moss)

Vaccinia virus recombinant protects chimpanzees against hepatitis B. A vaccinia virus recombinant that expresses the hepatitis B virus surface antigen gene was constructed. To determine the ability of the recombinant virus to protect against disease, two chimpanzees received a single intradermal vaccination. A third chimpanzee, used as a control, was vaccinated with ordinary vaccinia virus. Fourteen weeks after vaccination, all 3 animals were challenged intravenously with live hepatitis B virus. The control chimpanzee developed typical hepatitis: circulating hepatitis surface antigen appeared 4 weeks later and elevated levels of alanine aminotransferase were found at 9 weeks. In contrast, neither of the chimpanzees vaccinated with recombinant virus had detectable surface antigen or biochemical evidence of hepatitis. The vaccination appeared to work by priming the immune system so that challenge with hepatitis B virus resulted in a mild inapparent infection that led to an anamnestic immune response. (Smith and Moss)

Vaccinia virus recombinant protects hamsters against influenza infection. A DNA copy of the influenza virus hemagglutinin gene was inserted into the genome of vaccinia virus under control of an early vaccinia virus promoter. Tissue culture cells infected with the purified recombinant virus synthesized influenza virus hemagglutinin which was glycosylated and transported to the cell surface. Vaccinated hamsters achieved levels of antibody similar to those obtained upon primary infection with influenza virus and were protected against lower respiratory infection with influenza virus. (Smith and Moss)

Vaccinia virus recombinant stimulates humoral and cell mediated immunity. For maximal effectiveness, a vaccine should produce cellular and humoral immunity. A vaccinia virus recombinant, that expresses the influenza virus hemagglutinin, was shown to prime and stimulate a specific murine cytotoxic T lymphocyte response. Histocompatible cells infected with this recombinant also serve as targets for cytotoxic T lymphocytes. (Smith and Moss)

Vaccinia virus recombinant expresses malaria antigen. The gene coding for the circumsporozoite antigen of *Plasmodium knowlesi* was inserted into the vaccinia virus genome under control of a vaccinia virus promoter. Cells infected with the recombinant virus synthesized polypeptides of 53,000 to 56,000 daltons that reacted with monoclonal antibody against the repeating epitope of the malaria protein. The polypeptide made by vaccinia virus was slightly larger than that obtained from the salivary gland of plasmodium infected mosquitoes suggesting that the leader segment was not cleaved off in mammalian cells. Rabbits that were vaccinated with the recombinant virus produced antibodies that bound specifically to sporozoites. These data provide the first evidence for expression of a cloned malaria gene in mammalian cells and illustrate the potential of vaccinia virus recombinants as malaria vaccines. (Smith and Moss)

Regulation of late vaccinia virus genes. A subset of vaccinia virus genes are expressed only after DNA replication. To investigate the regulation of such transcriptional units, a representative gene encoding a major late polypeptide of Mr 28,000 was mapped and sequenced. Translatable mRNAs were heterogeneous in length and overlapped several early genes downstream. The 5' end of the message was located and the DNA segment upstream was excised and ligated to the prokaryotic chloramphenicol acetyltransferase gene. The resulting chimeric gene was recombined into the thymidine kinase locus of the vaccinia virus genome and infectious recombinant virus was isolated. Both the time of enzyme synthesis in infected cells and the requirement for DNA replication indicated that the sequence upstream of the late gene contains cis-acting transcriptional regulatory sequences. (Weir and Moss)

Selective transcription of vaccinia virus genes in template dependent soluble extracts of infected cells. A soluble system prepared from lysates of vaccinia virus infected cells specifically and accurately initiates transcription on defined vaccinia virus templates. The required regulatory signals are contained within a DNA segment extending about 230 base pairs upstream and 30 bases downstream of the RNA start site. Transcription is resistant to α -amanitin and inhibited by antibodies to the viral RNA polymerase. Whole cell extracts from uninfected cells cannot accurately transcribe vaccinia DNA. Conversely, extracts prepared at 2 hours or later after vaccinia virus infection no longer transcribe RNA polymerase II templates. These profound changes in transcriptional specificity may contribute to the selective expression of viral genes following vaccinia infection.

(Puckett and Moss)

Transient assay system for analysis of vaccinia virus gene expression. Recombinant plasmids containing the promoter regions of vaccinia virus genes ligated to the coding segment of the chloramphenicol acetyltransferase gene, were constructed. When the plasmids were introduced by transfection into vaccinia virus infected cells, the chimeric gene was expressed and significant levels of enzyme accumulated. Enzyme was not detected when the same recombinant plasmid was introduced into uninfected cells. Nor was activity detected when the vaccinia virus promoter was absent from the plasmid or was replaced by SV40 or Rous sarcoma virus promoters. This specificity indicated that expression is dependent on a cis-acting vaccinia virus promoter region within the recombinant plasmid and diffusible trans-acting transcription factors produced during virus infection. (Cochran and Moss)

The DNA polymerase gene of vaccinia virus has been physically mapped. The genetic locus responsible for resistance to phosphonoacetic acid was mapped within the HindIII E fragment of the vaccinia virus genome. To prove that this is the structural gene for DNA polymerase, mRNA was hybridized to cloned subfragments of HindIII E and then translated in a reticulocyte cell-free system. One product was a polypeptide of about 100,000 daltons that co-electrophoresed with purified vaccinia virus DNA polymerase. Polyacrylamide gel electrophoresis of protease digested cell-free translation product and purified enzyme confirmed their identity. The 5' and 3' ends of the mRNA were mapped by Northern blotting and by nuclease S1 digestion. Two overlapping transcripts differing in their 3' ends were identified. Portions of the DNA polymerase gene were sequenced. (Jones and Moss)

Cloning of the vaccinia virus telomere. An XbaI fragment containing the terminal hairpin loop of vaccinia virus DNA was purified. The fragment was denatured and then hybridized to form a duplex structure which was cloned in the plasmid vector pUC13. Restriction endonuclease digestion and nucleotide sequencing confirmed that the duplex was a palindrome-like structure derived from the hairpin loop. The cloned DNA fragment should provide large amounts of DNA for physical and enzymatic studies. (Moss)

Effects of aphidicolin on vaccinia virus. Vaccinia virus yield and plaque formation were reduced several hundred fold by 80 μ M aphidicolin. Viral and cellular DNA synthesis were reduced when infected cells were incubated with the drug. A mutant vaccinia virus which is resistant to aphidicolin was isolated and developed by passaging in cells in 80 μ M aphidicolin. The yield of the resistant virus was several hundred fold greater than the yield of the wild-type parent, and synthesis of the DNA of the drug-resistant virus was over four times greater than the synthesis of wild-type viral DNA, in the presence of 80 μ M aphidicolin. Viral DNA polymerase isolated from cells infected with resistant virus was more resistant to aphidicolin than the viral DNA polymerase isolated from cells with wild-type virus when both enzymes were tested in an *in vitro* assay. The implication is that an altered viral polymerase is responsible for the drug resistance of the mutant virus. Intact DNA isolated from the drug-resistant virus was able to rescue aphidicolin-sensitive vaccinia virus in cells incubated in 80 μ M aphidicolin. Since the viral DNA polymerase gene is thought to be in the Hind-E segment of the viral DNA, this segment and others have been cloned in a pUC plasmid. (DeFilippes)

Ectromelia virus, an orthopoxvirus, causes a natural infection (mousepox) in colonized mice. We have studied the basic biology of the virus as it relates to surveillance and control measures employed in mouse colonies. Our results indicate that immunizing mice with the vaccinia virus IHD-T strain is ineffective at preventing transmission of ectromelia virus in a mouse colony. Certain mouse-derived cell lines such as hybridomas and lymphomas were shown capable of supporting ectromelia virus replication in vitro, and thus could be a factor in the introduction of mousepox into a mouse colony. The genotype of the mouse was determined to be an important factor in determining whether mousepox was an apparent or inapparent infection; specifically the C57BL/6 mouse was shown to have a dominant autosomal locus at least partially responsible for reduced mortality rate from mousepox.

Recently initiated studies examining the molecular basis of orthopoxvirus pathogenesis have resulted in the characterization of a number of mutants which have altered pathogenicity in inbred mice. The molecular basis of those mutations and their correlation to virus virulence is the major direction of current research. These studies should directly contribute to the development of safe, effective recombinant orthopoxvirus vaccines for animal and human use. (Buller, Wallace)

Honors and Awards

Dr. Bernard Moss is an editor of the Journal of Biological Chemistry and is on the advisory board of Advances in Virus Research. He also serves on the editorial boards of the Journal of Virology, Virology, Intervirology and Antimicrobial Agents and Chemotherapy. Dr. Moss is a member of the Advisory Committee on Nucleic Acid and Protein Synthesis of the American Cancer Society and served as an advisor to the World Health Organization. During the past year Dr. Moss received the Becton-Dickinson Traveling Lecture Award, received the Public Health Service Meritorious Service Medal, was on the guest faculty of the University of California, San Francisco and of Johns Hopkins Medical School, and chaired the workshop on cytoplasmic animal and insect DNA viruses at the 6th International Congress of Virology.

Dr. Frank DeFilippes served on the thesis panel for D. Moynet at the University of Toulouse.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00011-19 LVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of small DNA containing viruses belonging to the family Parvoviridae

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. David Hoggan Senior Investigator LVD, NIAID

Others: S. I. Benn Medical Staff Fellow LVD, NIAID

COOPERATING UNITS (if any)

K. I. Berns, Univ. Florida, College of Medicine, Gainesville, Florida
N. R. Blacklow, Univ. Massachusetts Medical Center, Worcester, Massachusetts
R. L. Wilder, A&R, NIADKD

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

.50

PROFESSIONAL

.20

OTHER:

.3

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Work has continued at low level. The isolation of a parvovirus from human synovial tissues and fluids has stimulated a survey of such tissues for the presence of known parvovirus sequences in human and mouse pathological tissues using various parvovirus DNA clones as hybridization probes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00020-09 LVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Studies on the treatment of disease with the interferon system

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: H. B. Levy

Head, Molecular Virology Section

LVD, NIAID

COOPERATING UNITS (if any) Drs. Chirigos, Foon, Necheles, NCI; M. Droller, Johns Hopkins Hosp; Drs. Muggia & Levin, N.Y.U. CA Cntr; E. Borden, U. Wisconsin Med. Sch.; J. Reed, Portsmouth Naval Hosp; B. Lampkin, Childrens CA Testing Grp; A. Salazar, Walter Reed; D. McFarlin, NINCDS; W. Engel, USC; R. Tyndall, U. Texas Med. Sch.

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Molecular Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1

PROFESSIONAL:

1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects

☐ (b) Human tissues

☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In tests done with the Biologic Response Modifier Program (BRMP) of the N.C.I., Poly ICLC has been found to be the most effective B.R.M. that has been tested there. It augments MLR reaction, cytotoxic macrophages, specific cytotoxic T lymphocytes and other immune functions. When used in conjunction with a non effective tumor vaccine, it leads to the development of specific immunity to that tumor. It is effective vs. a variety of metastatic and spontaneous tumors in mice.

There have been found 2/8 partial remissions in Renal Cell Carcinoma and 2/10 partial remissions in malignant melanoma in humans--both very resistant tumors. Negative results have been found in leukemia, neuroblastoma and metastatic breast cancer.

Additional studies were peripheral neuropathies (at Walter Reed and Texas Medical School, Dallas, Texas) have shown 4/4 improvements. Ten patients with multiple sclerosis in a chronic declining phase have been on a study for upward of 10 months. Eight have stabilized or improved, 2 have worsened. These patients show a variety of changes in immune related functions.

| | | | | | | | | | | | |
|--|------------------------------|--|---------------------|--------------|------------|-------------------------------|--------------|------------|----------------|------------------|------------|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00123-18 LVD | | | | | | | | | |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Structure and replication of poxvirus genomes | | | | | | | | | | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: E. Jones</td> <td style="width: 33%;">Staff Fellow</td> <td style="width: 33%;">LVD, NIAID</td> </tr> <tr> <td>Others: M. Merchlinsky</td> <td>Staff Fellow</td> <td>LVD, NIAID</td> </tr> <tr> <td>B. Moss</td> <td>Medical Director</td> <td>LVD, NIAID</td> </tr> </table> | | | PI: E. Jones | Staff Fellow | LVD, NIAID | Others: M. Merchlinsky | Staff Fellow | LVD, NIAID | B. Moss | Medical Director | LVD, NIAID |
| PI: E. Jones | Staff Fellow | LVD, NIAID | | | | | | | | | |
| Others: M. Merchlinsky | Staff Fellow | LVD, NIAID | | | | | | | | | |
| B. Moss | Medical Director | LVD, NIAID | | | | | | | | | |
| COOPERATING UNITS (if any) | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Viral Diseases | | | | | | | | | | | |
| SECTION Macromolecular Biology Section | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland | | | | | | | | | | | |
| TOTAL MAN-YEARS: 2.35 | PROFESSIONAL: 1.90 | OTHER: 0.45 | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | | | | | | | | | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Poxviruses are large DNA viruses that replicate in the cytoplasm of infected cells. Vaccinia virus, the prototype for this group, has a 180,000 base pair linear double-stranded DNA genome with covalently linked ends and a 10,000 base pair inverted terminal repetition. Nucleotide sequence analysis revealed that the ends of the genome consist of hairpin loops so that the two DNA strands form one continuous polynucleotide chain. The 104 nucleotide apex of the loop is rich in adenosine and thymidine nucleotides, incompletely base-paired and exists in two isomeric forms that are inverted and complementary in sequence (flip-flopped). During replication, head to head concatemers are formed. Models for the replication of the terminal segment of the poxvirus genome that involve site specific nicking and lead to flip-flop rearrangements and head to head dimerization have been proposed. Enzymes involved in DNA replication are encoded within the vaccinia virus genome. Using phosphonoacetate resistance as a genetic marker, the DNA polymerase gene has been precisely mapped. Hybridization of mRNA, cell-free translation, and peptide mapping confirmed the location of the DNA polymerase gene. </p> | | | | | | | | | | | |

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00126-11 LVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders)

Functional Analysis of Vaccinia Virus DNA

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: F. M. Defilippes

Research Physicist

LVD, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Macromolecular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

1.3

PROFESSIONAL

1

OTHER

.3

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Vaccinia virus growth in BSC-1 and HeLa cells was inhibited by aphidicolin concentrations of 20 μ M or more. Virus yield and plaque formation in BSC-1 cells were reduced several hundredfold by 80 μ M aphidicolin. The yield was decreased when drug was added early in infection, but was not significantly affected when drug was added at 7.5 h post-infection. Viral inhibition was completely reversed by removing the drug and suspending the infected cells in drug-free medium. DNA synthesis in uninfected cells was reduced about tenfold by 1 μ M aphidicolin. In cells with replicating vaccinia virus DNA, aphidicolin concentrations over 10 μ M were needed to reduce DNA synthesis to the same extent as in uninfected cells. Aphidicolin inhibition of viral DNA synthesis is removed by washing and suspending infected cells in drug-free medium. Fractionation of infected cells which had been incubated with 1 μ M drug showed that cytoplasmic viral DNA synthesis was relatively resistant to this aphidicolin concentration. The radioactivity associated with crude nuclei from these cells was estimated to be mainly from vaccinia DNA synthesis. Spontaneous virus mutants which were resistant to 60 or 80 μ M aphidicolin did not appear. However, after mutagenesis with hydroxylamine, mutants were generated which did form large plaques in medium with 80 μ M drug. In cells with replicating aphidicolin-resistant virus, DNA synthesis was more than four times more resistant to 80 μ M aphidicolin than in cells with replicating wild-type virus. The chromatographic patterns of the viral DNA polymerase isolated from cells with replicating wild-type or resistant virus were similar. However, in an *in vitro* DNA polymerase assay, 50% inhibition of enzyme activity was obtained with about 75 and 188 μ M aphidicolin for the wild-type and resistant polymerases, respectively. The viral enzymes were much more resistant to the drug than were the cell polymerases. Both intact and Hind-3 digested DNA from the drug-resistant virus were able to rescue aphidicolin-sensitive virus. The resistant DNA has been cloned into a bacterial plasmid.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00135-10 LVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Properties of immunoglobulin secreting cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---------|-------------------|---|------------|
| PI: | H. C. Morse III | Head, Virology & Cellular Immunology Sec. | LVD, NIAID |
| Others: | W. F. Davidson | Visiting Scientist | LVD, NIAID |
| | T. N. Fredrickson | Research Microbiologist | LVD, NIAID |
| | J. W. Hartley | Senior Investigator | LVD, NIAID |
| | R. L. Coffman | Senior Investigator, DNAX Res. Inst., Palo Alto, CA | |
| | M. C. Lamers | Senior Investigator | I, C |
| | K. L. Holmes | Staff Fellow | LVD, NIAID |
| | E. Rudikoff | Biological Laboratory Technician | LVD, NIAID |

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Virology and Cellular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:

1.25

PROFESSIONAL:

0.75

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
 ☐ (b) Human tissues
 ☒ (c) Neither
- ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Lymphomas of the B lymphocyte lineage have been studied in a number of systems. They occur in high frequency in NFS mice congenic for ecotropic murine leukemia virus induction loci of AKR and C58 mice. These lymphomas can be transplanted and grown as continuous *in vitro* lines. They include mature B cell, pre-B cell, and what has been termed pro-B cell lymphomas. Some lymphomas of each type express the cell surface antigen Ly-1 and one lymphoma was found to produce autoantibodies to bromelain-treated mouse red blood cells. Pro-B cells appear to be the *in vivo* target cell for both Abelson and Cas-NS-1 transforming viruses.

Continuous lines of non-transformed B cells have been established in culture. The cells are uniformly Ly-1⁺ and express λ light chains. Their growth is completely inhibited by treatment with anti-IgM antibodies.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00138-10 LVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders)

Viruses and the immune response

PRINCIPAL INVESTIGATOR (List either professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---------|-------------------|---|------------|
| PI | H. C. Morse III | Head, Virology & Cellular Immunology Sec. | LVD, NIAID |
| Others: | J. W. Hartley | Research Microbiologist | LVD, NIAID |
| | R. A. Yetter | Guest Worker | LVD, NIAID |
| | W. Y. Langdon | Visiting Fellow | LVD, NIAID |
| | T. N. Fredrickson | Research Microbiologist | LVD, NIAID |
| | J. N. Ihle | Senior Investigator | FCRC, NCI |
| | C. E. Buckler | Research Biologist | LVD, NIAID |
| | T. S. Theodore | Senior Investigator | LMM, NIAID |
| | M. A. Martin | Senior Investigator | LMM, NIAID |
| | K. L. Holmes | Staff Fellow | LVD, NIAID |

COOPERATING UNITS (if any)

P. M. Hoffman, V.A. Research Service at the University of Maryland, Baltimore
J. H. Stimpfling, McLaughlin Research Institute, Great Falls, Montana

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Virology and Cellular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland

TOTAL MAN-YEARS

3.0

PROFESSIONAL

2.0

OTHER

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Replication competent Cas-Br-M ecotropic murine leukemia virus (MuLV) induces a wide spectrum of hematopoietic neoplasms after infection of newborn mice. They include T and B cell lymphomas, myelogenous leukemias and erythroleukemias. The mechanisms responsible for this diversity of effects were shown to include the secondary production of lineage specific MuLV as well as effects of interleukin 3 (Il 3). The lineage specific viruses were a) a new transforming virus for murine fibroblasts and pre-B cells, termed Cas-NS-1, and b) a mink lung cell focus-forming (MCF) MuLV, termed Cas-NS-6. Continuous cell lines were established in the presence but not the absence of Il 3 from spleens of mice with lymphomas induced by Cas-Br-M.

A new mutation to ashen in B10.F mice was found to be associated with a new germ line integration of an ecotropic provirus. Genetic studies of the mutant mice showed that the coat color mutation and virus reintegration were independent events.

Studies of thymic lymphomas induced in AKR mice by either of two MCF MuLV were markedly heterogeneous in their cell-surface antigenic phenotypes. Single MCF MuLV therefore do not predictably induce neoplasms of an antigenically distinct subset of thymocytes.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00205-04 LVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Abnormalities of T and B lymphocytes of autoimmune mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|-----|-----------------|---------------------|------------|
| PI: | W. F. Davidson | Visiting Scientist | LVD, NIAID |
| | H. C. Morse III | Senior Investigator | LVD, NIAID |

| | | | |
|---------|-------------------|----------------------------------|------------|
| Others: | J. W. Hartley | Senior Investigator | LVD, NIAID |
| | T. N. Fredrickson | Research Microbiologist | LVD, NIAID |
| | E. Rudikoff | Biological Laboratory Technician | LVD, NIAID |

COOPERATING UNITS (if any)

J. B. Roths, Jackson Laboratory, Bar Harbor, ME

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Virology and Cellular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

1.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- | | | |
|---|--|---|
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input checked="" type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors | | |
| <input type="checkbox"/> (a2) Interviews | | |

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Lymphocytes from mice with lymphoproliferation due to the effects of the nonallelic autosomal recessive mutations lpr (lymphoproliferation) and gld (generalized lymphoproliferative disease) were studied for a series of phenotypic and functional abnormalities. Both mutations had been implicated in the development of autoimmune disease as a result of undetermined mechanisms, although it had been suggested that polyclonal B cell activation and/or deficient production of interleukin 2 (IL 2) was responsible for renal disease in lpr homozygotes. Studies of C3H mice homozygous for the lpr mutation showed that they were deficient in their ability to produce IL 2 in vitro and exhibited polyclonal B cell activation but did not develop renal disease. These results indicate that neither of these abnormalities alone or in combination is sufficient cause for the development of lupus-like renal disease.

Studies of C3H-gld/gld mice showed that they had multiple phenotypic and functional abnormalities in common with lpr homozygotes including polyclonal B cell activation, deficient production of IL 2 and expansion of a unique subset of lymphocytes. These results suggest that lpr and gld may be mutations in different enzymes that act in a common metabolic pathway of importance to the differentiation and function of T lymphocytes.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00280-03 LVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Ecotropic murine leukemia virus proviral sequences in inbred mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: C. E. Buckler

Senior Investigator

LVD, NIAID

COOPERATING UNITS (if any)

R. Epstein, The Salk Institute

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

.25

PROFESSIONAL

OTHER

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Close linkage was found to exist between Akv-2, an ecotropic provirus found uniquely on chromosome 16 of AKR/N mice, and the immunoglobulin λ_1 light chain locus, Igl-1. No recombinants between the Igl-1 locus and Akv-2 were found by Southern blot analysis of DNA obtained from progeny of the backcross of (AKR/N x SJL/J) F_1 to SJL/J, indicating that these genes map within 5.9 cM of each other. A probe specific for the flanking sequence of Akv-2 was used to detect the provirus, while one specific for the Igl-1 constant region was used to determine which allele of the structural gene was expressed in the backcross mice. The constant region of Igl-1 differs between AKR/N and SJL/J with respect to a site for the restriction endonuclease KpnI.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00281-03 LVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular cloning of recombinant MuLV proviral sequences from AKR tumors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C. E. Buckler

Senior Investigator

LVD, NIAID

COOPERATING UNITS (if any)

T. Theodore and M. A. Martin, LMM, NIAID

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

0.75

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

DNA isolated from 19 MCF247 induced tumors in AKR/J mice was studied. A MuLV insert unique to one tumor was cloned from a tumor DNA library in lambda phage. A flanking sequence probe for this cloned tumor MuLV insert was constructed in pBR322 and used to evaluate the other 18 tumors for indications of a common region of MuLV insertion. No evidence for such conditions were obtained indicating that the cloned, tumor unique MuLV may be unrelated to thymoma induction. Isolation of additional MuLV inserts from the tumor DNA library is in progress to continue the study.

Evaluation of the 19 tumor DNAs by Southern blot analysis showed that none contained c-myc polymorphisms. Similar studies with other onc probes are in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00282-01 LVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Malignant lymphomas occurring in congenic mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: T. N. Fredrickson

Research Microbiologist

LVD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland

TOTAL MAN-YEARS

PROFESSIONAL

OTHER

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type Do not exceed the space provided)

Terminated.

Continuing aspects of this project are included in Z01 AI 00135-10 LVD.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00283-01 LVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The pathogenesis of chronic myelogenous leukemia in NFS/N mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: T. N. Fredrickson

Research Microbiologist

LVD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Terminated.

Continuing aspects of this project are included in Z01 AI 00284-02 LVD.

| | | |
|--|--------------------|---------------------------------------|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00284-03 LVD |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Characterization of pathogenic murine leukemia viruses | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: J. W. Hartley Head, Viral Oncology Section LVD, NIAID | | |
| Others: H. C. Morse Senior Investigator LVD, NIAID T. N. Fredrickson Research Microbiologist U. CT, LVD, NIAID W. Y. Langdon Guest Worker LVD, NIAID J. E. Silver Medical Staff Fellow LVD, NIAID T. S. Theodore Senior Investigator LMM, NIAID M. A. Martin Senior Investigator LMM, NIAID S. Ruscetti Senior Investigator LG, C | | |
| COOPERATING UNITS (if any) Nancy Hopkins, MIT | | |
| LAB/BRANCH Laboratory of Viral Diseases | | |
| SECTION Viral Oncology Section | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MAN-YEARS 2.5 | PROFESSIONAL .8 | OTHER 1.8 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <p>The pathogenicity for mice of ecotropic and MCF-type recombinant murine C-type viruses (MuLV) and of recombinant viruses constructed <u>in vitro</u> is under study. Two ecotropic viruses from wild mice, C2S-M and CasBr-M, have been found to induce an unusual array of hematopoietic neoplasms in NFS mice inoculated as newborns. Several replication competent MCF viruses as well as two defective rapidly oncogenic viruses have been isolated from tumors of various types and are being characterized for pathogenicity, effects of host genetic influences, and restriction enzyme patterns.</p> <p>For studies designed to test directly the role of those portions of MuLV genomes which have been found to differ regularly between viruses with differing capacities to induce disease, <u>in vitro</u> recombinants were prepared from restriction enzyme fragments of molecularly cloned leukemogenic AKR MCF 247 virus and non-leukemogenic AKR ecotropic virus. Similarly, recombinant viruses were derived from cloned Moloney MuLV, which induces lymphoblastic lymphoma, and Friend MuLV, which induces erythroleukemia. Results of pathogenicity testing of these recombinant viruses indicate that control signals in the LTR region of the genome are critical in determining target cell-specificity in the Friend-Moloney system, and play a role in MCF 247-induced acceleration of AKR thymic lymphoma. In the latter case, nucleotide sequences in the <u>env</u> region, including segments coding for both gp70 and p15E, also contribute significantly to the leukemogenic phenotype.</p> | | |

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00286-03 LVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of genetic control of murine leukemia viruses and virus-induced neoplasms

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---------|-------------------|------------------------------|-------------------|
| PI: | J. W. Hartley | Head, Viral Oncology Section | LVD, NIAID |
| Others: | H. C. Morse | Senior Investigator | LVD, NIAID |
| | T. N. Fredrickson | Research Microbiologist | U. CT; LVD, NIAID |
| | M. Potter | Senior Investigator | LG, C |

COOPERATING UNITS (if any)

Nancy Hopkins, MIT
F. Becker, M.D. Anderson Hospital & Tumor Institute

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:

2.2

PROFESSIONAL:

.4

OTHER:

1.8

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies of the host gene $Rmcf^r$, which specifically depresses in vitro and in vivo replication of MCF viruses, indicate that while this allele can suppress spontaneous and AKR MCF-induced thymic lymphoma in crosses of AKR and $Rmcf^r$ mouse strains, and results in lowered frequency and extended latent periods for Friend virus-induced erythroleukemia, it is much less effective in controlling T-cell lymphomas caused by Moloney or Gross Passage A virus. $Rmcf$ alleles have been shown not to affect pristane-induced plasmacytomas in BALB/c mice.

As part of a long-term program to analyze the effects of murine retrovirus associated genes on hematopoietic system tumors, both spontaneous and those induced by chemicals or virus inoculation, a number of genes of interest are being bred onto inbred mouse backgrounds. The majority of congenic lines are being established on the NFS Swiss mouse background, providing a standard background which is negative for its own endogenous ecotropic virus. Lines include the V-loci congenics (carrying ecotropic virus loci from AKR, C58, or C3H/Fg; and mice carrying specific viral resistance genes (FV-1, $Rmcf$) or linkage marker genes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00287-03 LVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Chromosome mapping of xenotropic MuLV DNA and protooncogenes in inbred mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. David Hoggan

Senior Investigator

LVD, NIAID

Others: C. A. Kozak

Senior Staff Fellow

LVD, NIAID

COOPERATING UNITS (if any)

M. A. Martin, LMM, NIAID

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

0.80

PROFESSIONAL:

.60

OTHER:

.20

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

The work on this project is largely completed and a number of protooncogenes have been mapped in the mouse.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00288-02 LVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular cloning of endogenous xenotropic MuLV proviral DNA

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. David Hoggan Senior Investigator LVD, NIAID

Others: C. E. Buckler Senior Investigator LVD, NIAID

COOPERATING UNITS (if any)

M. A. Martin and T. Theodore, LMM, NIAID

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

.2

PROFESSIONAL:

.1

OTHER:

.1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Current data suggests that xenotropic MuLV are not related to the genesis of any known disease we have therefore determined to terminate this project.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00289-02 LVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Organization of endogenous proviral DNAs of xenotropic murine leukemia viruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. David Hoggan

Senior Investigator

LVD, NIAID

Others: C. E. Buckler

Senior Investigator

LVD, NIAID

COOPERATING UNITS (if any)

M. A. Martin, LMM, NIAID

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

.1

PROFESSIONAL

.05

OTHER

.05

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type Do not exceed the space provided.)

Terminated.

| | | | | | | | | | | | | | | | | | |
|--|--|---|---|---|--------------------------------------|--------------------------------------|--|--|--|--------------------|------------|--------------|--------------|------------|----------|--------------------|------------|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER <div style="text-align: center; font-weight: bold;">Z01 AI 00298-03 LVD</div> | | | | | | | | | | | | | | | |
| PERIOD COVERED <div style="text-align: center; font-weight: bold;">October 1, 1983 to September 30, 1984</div> | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <div style="text-align: center; font-weight: bold;">Development of vaccinia virus as an expression vector for live vaccines</div> | | | | | | | | | | | | | | | | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: B. Moss</td> <td style="width: 33%;">Medical Director</td> <td style="width: 33%; text-align: right;">LVD, NIAID</td> </tr> <tr> <td colspan="3"> </td> </tr> <tr> <td>Others: G. L. Smith</td> <td>Visiting Associate</td> <td style="text-align: right;">LVD, NIAID</td> </tr> <tr> <td>K. Brechling</td> <td>Staff Fellow</td> <td style="text-align: right;">LVD, NIAID</td> </tr> <tr> <td>J. Rosel</td> <td>Guest Investigator</td> <td style="text-align: right;">LVD, NIAID</td> </tr> </table> | | | PI: B. Moss | Medical Director | LVD, NIAID | | | | Others: G. L. Smith | Visiting Associate | LVD, NIAID | K. Brechling | Staff Fellow | LVD, NIAID | J. Rosel | Guest Investigator | LVD, NIAID |
| PI: B. Moss | Medical Director | LVD, NIAID | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | |
| Others: G. L. Smith | Visiting Associate | LVD, NIAID | | | | | | | | | | | | | | | |
| K. Brechling | Staff Fellow | LVD, NIAID | | | | | | | | | | | | | | | |
| J. Rosel | Guest Investigator | LVD, NIAID | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) <div style="text-align: center;">A. Notkins, National Inst. of Dental Research; W. Wunner, Wistar Inst.</div> | | | | | | | | | | | | | | | | | |
| LAB/BRANCH <div style="text-align: center;">Laboratory of Viral Diseases</div> | | | | | | | | | | | | | | | | | |
| SECTION <div style="text-align: center;">Macromolecular Biology Section</div> | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION <div style="text-align: center;">NIAID, NIH, Bethesda, Maryland</div> | | | | | | | | | | | | | | | | | |
| TOTAL MAN-YEARS: <div style="text-align: center; font-weight: bold;">2.0</div> | PROFESSIONAL: <div style="text-align: center; font-weight: bold;">1.5</div> | OTHER: <div style="text-align: center; font-weight: bold;">0.5</div> | | | | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td><input type="checkbox"/> (a) Human subjects</td> <td><input checked="" type="checkbox"/> (b) Human tissues</td> <td><input type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td colspan="2"></td> </tr> <tr> <td><input type="checkbox"/> (a2) Interviews</td> <td colspan="2"></td> </tr> </table> | | | <input type="checkbox"/> (a) Human subjects | <input checked="" type="checkbox"/> (b) Human tissues | <input type="checkbox"/> (c) Neither | <input type="checkbox"/> (a1) Minors | | | <input type="checkbox"/> (a2) Interviews | | | | | | | | |
| <input type="checkbox"/> (a) Human subjects | <input checked="" type="checkbox"/> (b) Human tissues | <input type="checkbox"/> (c) Neither | | | | | | | | | | | | | | | |
| <input type="checkbox"/> (a1) Minors | | | | | | | | | | | | | | | | | |
| <input type="checkbox"/> (a2) Interviews | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Procedures have been developed for the use of vaccinia virus as a eukaryotic expression vector. A chimeric gene is formed by ligating vaccinia virus transcriptional regulatory signals to a foreign protein coding sequence. Homologous recombination is used to insert the chimeric gene into a non-essential region of the vaccinia virus genome. To facilitate the formation and isolation of recombinant virus, special plasmid vectors have been constructed which direct the insertion of the chimeric gene into the thymidine kinase locus. Recombinant virus is then selected on the basis of the thymidine kinase negative phenotype. The recombinant viruses produced in this manner are stable and have a wide host range for tissue culture cells and animals. At least 25,000 base pairs of DNA can be inserted into the vaccinia virus genome without destroying infectivity. To optimize expression, the prokaryotic gene encoding chloramphenicol acetyltransferase was inserted into vaccinia virus under control of different promoters. This system has been used to express genes from a variety of infectious agents including herpes simplex virus type 1, hepatitis B virus, influenza virus, vesicular stomatitis virus, rabies virus, and the malaria parasite Plasmodium knowlesi. The potential of these recombinant viruses as live vaccines is being evaluated in experimental animals.</p> | | | | | | | | | | | | | | | | | |

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|---|---------------|---------------------------------------|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00299-03 LVD |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Genetic control and mapping of endogenous proviruses | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) | | |
| PI: | C. Kozak | Senior Staff Fellow LVD, NIAID |
| Others: | H. Morse III | Senior Investigator LVD, NIAID |
| | J. Hartley | Senior Investigator LVD, NIAID |
| COOPERATING UNITS (if any) V. Morris, Univ. Western Ontario R. Callahan, NCI, NIH | | |
| LAB/BRANCH Laboratory of Viral Diseases | | |
| SECTION Viral Oncology Section | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland | | |
| TOTAL MAN-YEARS | PROFESSIONAL: | OTHER: |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Studies on the genetic transmission of endogenous murine retroviruses have led to the chromosomal mapping of more than 10 distinct loci. Most recently, genetic crosses have been used to position 2 of the 3 mouse mammary tumor proviral loci carried by BALB/c mice. Analysis of additional laboratory strains and various feral mouse populations have been undertaken to describe the stability and wild mouse origin of specific loci and to identify additional genetic factors that alter <u>in vivo</u> virus expression and susceptibility to exogenous virus. These studies have shown that inbred and wild mice carry loci for induction of xenotropic murine leukemia virus (MuLV) other than the <u>Bxv-1</u> locus carried by at least 6 of the older inbred strains. Such loci were identified in MA/MY and NZB/BLN mice and in a partially inbred strain of feral Japanese mice, <u>M.m. molossinus</u> . Studies on the endogenous ecotropic and xenotropic MuLV related proviral sequences found in a variety of feral mouse populations have shown that almost all lack ecotropic-MuLV related sequences and many lack xenotropic MuLV related sequences. However, MuLVs with ecotropic host range have been isolated from <u>M. hortulanus</u> , <u>M.m. castaneus</u> and <u>M.m. molossinus</u> . Southern blot hybridization data indicate that these viruses differ from ecotropic MuLVs of laboratory mice in hybridization properties and internal restriction maps. Cells of most wild mice also differ from inbred mice in susceptibility to exogenous infection. Genetic crosses show that susceptibility to xenotropic MuLV is controlled by a single chromosome 1 locus, <u>Sxv</u> . These studies also show that the feral mice <u>M.m. praetextus</u> and <u>M. spretus</u> also lack <u>Fv-1</u> restriction. Genetic crosses indicate that these mice carry novel alleles at <u>Fv-1</u> . | | |

| | | |
|---|---------------|---------------------------------------|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00300-03 LVD |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Genetic control of resistance to Friend virus in inbred wild mouse populations | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) | | |
| PI: | C. Kozak | Senior Staff Fellow LVD, NIAID |
| Others: | C. Buckler | Senior Investigator LVD, NIAID |
| | M. Hoggan | Senior Investigator LVD, NIAID |
| | H. Ikeda | Visiting Fellow LVD, NIAID |
| COOPERATING UNITS (if any) M. Potter, NCI, NIH; F. Laigret, LMI, NIAID; R. Repaske, OSD, NIAID, T. Theodore and M. Martin, LMM, NIAID | | |
| LAB/BRANCH Laboratory of Viral Diseases | | |
| SECTION Viral Oncology Section | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205 | | |
| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
| .83 | .42 | .41 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Various feral mice are resistant to induction of erythroblastosis by Friend complex virus. In the Japanese mouse, <i>M. m. molossinus</i>, resistance results from inheritance of the Fv-4 resistance locus. Sensitivity to Friend complex disease was examined in these mice and in BALB/c congenics carrying Fv-4^r in order to characterize Fv-4. Southern blot analysis showed that a novel ecotropic viral envelope related sequence is integrated at or near Fv-4 in resistant mice. This proviral sequence cannot be induced to produce infectious virus, but immunological methods have identified a cell surface antigen on thymocytes of Fv-4^r mice related to the ecotropic envelope glycoprotein. The Fv-4^r associated proviral sequence has now been molecularly cloned and biochemically characterized. The cloned fragment contains 4.0 kb of ecotropic MuLV sequences (pol, env, LTR) and cellular flanking sequences. The nucleotide sequence of 3 kb of the viral sequences indicates that the env region resembles other ecotropic MuLVs. These data are consistent with an interference model for Fv-4 resistance in which the Fv-4^r gene product binds to cell surface receptors used by ecotropic MuLVs. Other wild mice are also resistant to Friend complex disease, but lack the Fv-4 associated sequence. The resistance shown by <i>M. spretus</i> resembles that of Fv-2 resistant mice and results of crosses with Fv-2 sensitive and resistant inbred mice indicate that <i>M. spretus</i> carries the resistance allele at this locus. </p> | | |

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00301-03 LVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Somatic cell genetic studies on endogenous proviruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C. Kozak

Senior Staff Fellow

LVD, NIAID

Others: M. Hoggan

Senior Investigator

LVD, NIAID

COOPERATING UNITS (if any)

P. Pitha, Johns Hopkins; R. Callahan, D. Nebert, U. Rapp, S. O'Brien, NCI, NIH;
 G. Peters, ICRF; P. Tsichlis, Fox Chase Cancer Center, Philadelphia; R. Risser,
 McArdle Lab. for CA Res., Madison; P. Jolicoeur, Clinical Res. Inst., of Montreal.

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.2

PROFESSIONAL:

.6

OTHER:

.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Interspecific somatic cell hybrids have been extensively used to expand our studies on the genetic basis of tumorigenesis. We have used somatic cell hybrids to identify and chromosomally map specific genes involved in oncogenesis and to construct cell lines carrying isolated genetic elements of this complex multi-gene system for their further characterization. Most recent studies on viral receptor genes have mapped receptor loci for ecotropic and MCF MuLVs to different chromosomes. Hybrids which lack specific receptor loci were used to describe the time course of virus induction for different endogenous proviral loci. Finally, somatic cell hybrids are being analyzed by blot hybridization with molecularly cloned probes to describe the chromosomal localization of proviral and other cancer-related loci. These studies have resulted in the localization of endogenous mouse mammary tumor viral (MMTV) loci to 2 mouse chromosomes and tumor-specific MMTV integrations to 4 mouse chromosomes. Xenotropic envelope-reactive sequences were mapped to almost all of the mouse chromosomes and a single provirus associated with the Bxv-1 induction locus was identified. Three loci representing tumor-specific ecotropic MuLV integration were mapped to chromosome 15. Finally, analysis of hybrids has provided specific map locations for various proto-oncogenes and for interferon structural genes.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00302-02 LVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Congenic mice with genes of importance to murine leukemia virus infection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: W. P. Rowe

Chief, Laboratory of Viral Diseases

LVD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Terminated.

Continuing aspects of this project are included in Z01 AI 00286-03 LVD.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00303-02 LVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Genetic resistance to murine leukemia viruses and virus-induced neoplasms

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: W. P. Rowe

Chief

LVD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland

TOTAL MAN-YEARS

PROFESSIONAL

OTHER

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

Terminated.

Continuing aspects of this project are included in:

Z01 AI 00286-03 LVD

Z01 AI 00138-10 LVD

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00304-03 LVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Hematologic and genetic studies of malignancies induced by Friend helper virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J. E. Silver

Medical Staff Fellow

LVD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

2

PROFESSIONAL:

1

OTHER:

1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Friend helper virus (F-eco) is a murine retrovirus which can induce a variety of hematopoietic neoplasms. This virus is being used as a model system to study basic mechanisms of leukemogenesis.

1. Genetic studies. Crosses between AKR and other strains indicated that AKR mice carry at least two genes other than inherited ecotropic viruses which predispose them to lymphoma a few months after inoculation with F-eco. One of these genes may be on chromosome 15, a chromosome which carries an oncogene (myc) and virus integration sites (Mlvi-1 and Mlvi-2) associated with lymphoma.

2. Virological studies. MCF viruses were found in F-eco inoculated mice developing erythroblastosis. However, MCF viruses were not found by standard infectious center assays or blot hybridization techniques in lymphomas or myeloid leukemias induced by F-eco in C57BL mice. Thus, MCF viruses are not pathogenic intermediates in these neoplasms. When C57BL mice were inoculated with pseudotypic mixtures of F-eco plus F-MCF, the MCF viruses grew well but did not cause erythroblastosis.

3. Molecular studies. Virus-cell junction fragments are being cloned from F-eco induced myeloid leukemias in C57BL mice. These experiments are designed to look for common integration sites or cellular oncogenes associated with myeloid leukemias.

Significance. Host genes affecting type of leukemia induced by F-eco may elucidate steps in viral leukemogenesis. MCF viruses are not necessary for some types of F-eco induced neoplasms. Common integration sites in myeloid leukemias are being sought as candidate oncogenes involved in myeloid leukemogenesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00305-02 LVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Studies on the mechanism of Fv-1^b inhibition of AKR thymomagenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J. Silver

Medical Staff Fellow

LVD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS

PROFESSIONAL

OTHER

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

Terminated.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00306-03 LVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The biology of mousepox

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R. M. L. Buller
G. D. WallaceVisiting Associate
Senior InvestigatorLVD, NIAID
LVD, NIAID

COOPERATING UNITS (if any)

Dr. H. C. Morse III, LVD, NIAID; Dr. B. Moss, LBV, NIAID; Dr. Weinblatt, ATCC;
Dr. M. Potter, LCBGY, NCI

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

2

PROFESSIONAL:

1

OTHER:

1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Ectromelia, an orthopoxvirus, causes mousepox in colonized mice. Studies were carried out to characterize the basic biology of the virus in inbred mice in order: 1) to improve the surveillance and control measures necessary to prevent future mousepox epizootics in the mouse colonies used in biomedical research; and 2) to study the molecular basis of orthopoxvirus pathogenesis in inbred mice using ectromelia and vaccinia virus mutants, and to apply the acquired knowledge towards the development of a safe, effective recombinant vaccinia virus vaccine for human use.

1. Prevention of mousepox epizootics by immunization with vaccinia virus IHD-T (a serologically closely related orthopoxvirus) is widespread. Our experiments have shown that as early as 1 month after immunization with strain IHD-T, BALB/cByJ mice can be infected by ectromelia virus and subsequently shown to transmit virus to susceptible mice. Therefore, the use of this strain for routine immunization of mouse colonies is of limited value in eradication of mousepox. Certain mouse derived transplantable - tumor cell lines such as hybridomas, which were passed in mice infected with ectromelia virus, were shown to become contaminated with the virus, and the ectromelia infectivity was demonstrable even after 1 month in tissue culture. Thus, mouse-derived products improperly screened for ectromelia virus can introduce mousepox into a mouse colony.

2. A number of ectromelia and vaccinia virus mutants have been characterized which grow normally in tissue culture but are avirulent in BALB/cByJ mice. Identification of the DNA sequence and protein(s) which encode the mutation, and their effect on virus pathogenesis in inbred mice is currently under investigation. These studies are necessary in order to modulate the virulence of the recombinant vaccinia virus vaccines, such that the probability of post-immunization complications due to vaccinia virus (ie. post-vaccinial encephalitis) are reduced to an acceptable level, but host immunity is conferred to the heterologous target antigen. Ectromelia and vaccinia recombinants may show promise for a mouse vaccine.

| | | | | | | | | | | | |
|---|---|---|-----------------------|---------------------|-------------------|----------------|--|--|---|---|---|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00307-03 LVD | | | | | | | | | |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Regulation of vaccinia virus gene expression | | | | | | | | | | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%; vertical-align: top;"> PI: J. P. Weir </td> <td style="width: 33%; vertical-align: top;"> Staff Fellow </td> <td style="width: 33%; vertical-align: top;"> LVD, NIAID </td> </tr> <tr> <td colspan="3" style="padding-top: 10px;"> Others: </td> </tr> <tr> <td style="vertical-align: top;"> C. Puckett M. Cochran S. Chakrabarti J. Rose B. Moss </td> <td style="vertical-align: top;"> Medical Staff Fellow Visiting Associate Visiting Associate Guest Investigator Medical Director </td> <td style="vertical-align: top;"> LVD, NIAID LVD, NIAID LVD, NIAID LVD, NIAID LVD, NIAID </td> </tr> </table> | | | PI: J. P. Weir | Staff Fellow | LVD, NIAID | Others: | | | C. Puckett M. Cochran S. Chakrabarti J. Rose B. Moss | Medical Staff Fellow Visiting Associate Visiting Associate Guest Investigator Medical Director | LVD, NIAID LVD, NIAID LVD, NIAID LVD, NIAID LVD, NIAID |
| PI: J. P. Weir | Staff Fellow | LVD, NIAID | | | | | | | | | |
| Others: | | | | | | | | | | | |
| C. Puckett M. Cochran S. Chakrabarti J. Rose B. Moss | Medical Staff Fellow Visiting Associate Visiting Associate Guest Investigator Medical Director | LVD, NIAID LVD, NIAID LVD, NIAID LVD, NIAID LVD, NIAID | | | | | | | | | |
| COOPERATING UNITS (if any) | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Viral Diseases | | | | | | | | | | | |
| SECTION Macromolecular Biology Section | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland | | | | | | | | | | | |
| TOTAL MAN-YEARS: <div style="text-align: center; font-weight: bold;">4.0</div> | PROFESSIONAL: <div style="text-align: center; font-weight: bold;">3.5</div> | OTHER: <div style="text-align: center; font-weight: bold;">0.5</div> | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 40%;"> <input checked="" type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither </div> </div> | | | | | | | | | | | |
| SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <p>Vaccinia virus contains a genome of more than 180,000 base pairs that encodes approximately 200 polypeptides. These genes are expressed in a coordinated fashion so that some polypeptides are made before and others after DNA replication. A large number of early and late genes have been transcriptionally or translationally mapped and some of each class have been sequenced. The regions preceding the coding segment are extremely rich in adenosine and thymidine nucleotides and differ substantially from eukaryotic consensus sequences. These sequences have been identified as promoter regions by in vivo and in vitro experiments. The regulatory signals specifying early or late expression are closely linked to the promoter. Evidence for the existence of virus-specific trans-acting transcriptional factors has been demonstrated by use of vaccinia virus infected cells for transient expression of recombinant plasmids. These factors have been demonstrated directly in extracts of infected cells.</p> | | | | | | | | | | | |

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00375-01 LVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Diagnosing of murine hematopathology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: T. N. Fredrickson

Research Microbiologist

LVD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Terminated.

Continuing aspects of this project are included in:

Z01 AI 00138-10 LVD
00135-10 LVD
00284-03 LVD
00286-03 LVD

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00376-02 LVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Serum survey of parvovirus antibody in normal and at risk AIDS populations.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---------|-----------------|----------------------|------------|
| PI: | M. David Hoggan | Senior Investigator | LVD, NIAID |
| Others: | S. I. Benn | Medical Staff Fellow | LVD, NIAID |
| | R. M. L. Buller | Visiting Associate | LVD, NIAID |

COOPERATING UNITS (if any)

M. A. Martin, LMM, NIAID

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

1.65

PROFESSIONAL:

1.45

OTHER:

.25

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Until recently the etiology of AIDS remained a mystery, although a number of viral agents have been shown to elucidate immunosuppressive activity in experimental animals. These include specific strains of the parvovirus, minute virus of mice, and to a lesser degree the recent canine parvovirus. It was therefore deemed of worth to search out any correlations between these agents and AIDS. An examination of greater than 25 sera from AIDS patients with specific ELISA to each of 10 distinct parvoviruses, which have been isolated from naturally occurring infections of canine, feline, bovine, porcine, murine, rat and human hosts, showed no significant correlation between AIDS and antibody to the test parvovirus. Furthermore, serum from 10 hemophiliacs, who are at a greater risk of getting AIDS, showed no abnormal presence of parvovirus antibodies. In addition, DNA was prepared from sera, semen, as well as various tissues from AIDS cases taken at autopsy. The DNA was negative when probed for parvovirus sequences from the forementioned parvovirus isolates.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00377-01 LVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Survey of SAIDS tissues and fluids for parvovirus antigens and antibody

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. David Hoggan Senior Investigator LVD, NIAID

Others: S. I. Benn Medical Staff Fellow LVD, NIAID

COOPERATING UNITS (if any)

M. Gardner, Regional Primate Center, Davis, California

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

.20

PROFESSIONAL:

.20

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Because the cause of SAIDS has been determined to be related to D-type retro-viruses and because the results of all our studies linking parvovriuses to SAIDS were negative this project has been terminated.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00391-01 LVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Expression of influenza virus genes by vaccinia virus recombinants

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: G. L. Smith Visiting Associate LVD, NIAID

Other: B. Moss Medical Director LVD, NIAID

COOPERATING UNITS (if any)

B. Murphy, Respiratory Diseases Section, LID, NIAID; J.R. Bennink and J.W. Yewdell,
The Wistar Institute

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Macromolecular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland

TOTAL MAN-YEARS

0.9

PROFESSIONAL

0.45

OTHER:

0.45

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A chimeric gene consisting of a vaccinia virus promoter linked to a cDNA copy of the influenza virus hemagglutinin gene was constructed and inserted into the vaccinia virus genome. Live recombinant virus was isolated and plaque purified. Tissue culture cells infected with the virus synthesized influenza virus hemagglutinin which was glycosylated and transported to the plasma membrane. Following vaccination, experimental animals produced circulating antibodies to the hemagglutinin and cytotoxic T lymphocytes that lysed target cells infected with influenza virus. Hamsters, that received a single intradermal vaccination were protected against influenza respiratory infection.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00392-01 LVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Expression of hepatitis B virus genes by vaccinia virus recombinants

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: G. L. Smith Visiting Associate LVD, NIAID

Others: K. Brechling Staff Fellow LVD, NIAID
B. Moss Medical Director LVD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Macromolecular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:

1.2

PROFESSIONAL:

.75

OTHER:

.45

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Hepatitis is a serious world-wide health problem. Approximately 200 million people are chronically infected with hepatitis B virus and large numbers of deaths are attributed to fulminant hepatitis, cirrhosis, and hepatocellular carcinoma. Although an effective subunit vaccine has been produced, limitations in supply and expense have prevented its global use. As an alternative, we are trying to construct a live recombinant hepatitis B vaccine. The gene for hepatitis B virus surface antigen has been engineered and inserted into the genome of vaccinia virus. The recombinant vaccinia virus is stable and expresses high levels of the hepatitis virus protein. The latter is glycosylated, assembled into particles and transported through the plasma membrane of infected cells. Rabbits, vaccinated with the recombinant virus, produce a high and sustained specific antibody response. Vaccination of chimpanzees resulted in priming of the immune system and protection against clinical hepatitis upon subsequent challenge with hepatitis B virus.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00393-01 LVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Expression of malaria genes by vaccinia virus recombinants

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: B. Moss

Medical Director

LVD, NIAID

Other: G. L. Smith

Visiting Associate

LVD, NIAID

COOPERATING UNITS (if any)

R. Nussenzweig, Department of Microbiology, NYU School of Medicine;
 V. Nussenzweig, Department of Pathology, NYU School of Medicine;
 N. Godson, Department of Biochemistry, NYU School of Medicine.

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Macromolecular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland

TOTAL MAN-YEARS

0.95

PROFESSIONAL

0.5

OTHER:

0.45

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☒ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Malaria remains a serious global health problem for which there is no effective vaccine. Previous studies indicate that animals can be immunized with inactivated sporozoites. The gene coding for the circumsporozoite antigen of the malaria parasite *Plasmodium knowlesi* was inserted into the vaccinia virus genome under the control of a defined vaccinia virus promoter. Tissue culture cells infected with the recombinant synthesized polypeptides that reacted with monoclonal antibody against the malaria protein. Rabbits vaccinated with the recombinant virus produced antibodies that bound specifically to sporozoites. These data provide evidence for expression of a cloned malaria gene in mammalian cells and illustrate the potential of vaccinia virus recombinants as malaria vaccines.

| | | |
|---|-----------------------------|--|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00416-01 LVD |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Recombinant vaccines against retroviruses associated with leukemia and AIDS | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI : B. Moss Medical Director LVD, NIAID Others: P. Earl Senior Staff Fellow LVD, NIAID G. Smith Staff Fellow LVD, NIAID | | |
| COOPERATING UNITS (if any) B. Chesebro, LPVD, NIAID; M. Martin, LMM, NIAID; P. Marx, California Primate Center; M. Gardner, University of California, Davis; J. Nunberg, Cetus. | | |
| LAB/BRANCH Laboratory of Viral Diseases | | |
| SECTION Macromolecular Biology Section | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland | | |
| TOTAL MAN-YEARS: 0.4 | PROFESSIONAL: 0.2 | OTHER: 0.6 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Retroviruses, long associated with leukemias and sarcomas of animals, have recently been implicated as the etiological agent of simian acquired immune deficiency syndrome (SAIDS), human T cell leukemias, and human acquired immune deficiency syndrome (AIDS). The identification of these agents makes it possible to consider various ways of prevention. The most promising approach is development of a vaccine that could be administered to individuals at risk. The vaccinia vector system will be evaluated because it has been shown to produce both humoral and cell mediated immunity against a variety of infectious agents. Since human retroviruses have not yet been shown to produce disease in animals, initial vaccine work must be done with animal retroviruses. Friend leukemia virus complex is a useful model system since it produces an acute disease in adult mice which can be prevented by repeated immunization with the envelope glycoprotein. The envelope glycoprotein gene has been isolated and is being engineered for expression in a live vaccinia virus vector. The ability of this recombinant vaccine to protect mice will be examined. The vaccinia virus vector system has been supplied to Cetus for development of a vaccine against feline leukemia virus, which causes immune deficiency disease in cats. Similarly, the envelope glycoprotein gene of SAIDS virus is being isolated for expression in vaccinia virus. The envelope glycoprotein gene of human AIDS viruses (HTLVIII/LAV) will be used when it becomes available. | | |

LABORATORY OF MICROBIAL STRUCTURE AND FUNCTION
Rocky Mountain Laboratories
Hamilton, Montana
1984 Annual Report
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Annual Report
Laboratory of Microbial Structure and Function
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1983, to September 30, 1984

RESEARCH HIGHLIGHTS

Major emphasis in LMSF is on structural and functional aspects of bacterial surface components that influence pathogenic organisms' interactions with the human and/or animal hosts. Diverse bacterial pathogens are studied including both extracellular and obligate intracellular agents of disease. Special attention is being paid to the variability in particular surface components found among differing strains and intrastrain variants of several bacteria including gonococci, relapsing fever and Lyme disease-causing spirochetes, salmonellae and other enterobacteria, contagious equine metritis organism, chlamydiae, spotted fever rickettsiae, and the rickettsia-like agents of Q fever. In addition to past and current emphases on biochemical and immunochemical dissection and characterization of bacterial membrane proteins and lipopolysaccharides, molecular genetic methods have attained appreciably more attention of LMSF staff members during the reporting period. Molecular cloning techniques and other DNA chemistry methodologies are being utilized to answer both biochemical and genetic control questions about bacterial surface components. Such genetic approaches are possible because of phenomenological and immunochemical features of bacteria and their surfaces that were previously established as the fruit of LMSF staff scientists' efforts. A few of the more noteworthy findings deriving from the past year's endeavors are summarized as follows:

Gonococci: Three surface protein constituents of gonococci (Gc)--pili, protein I, and protein II--have been intensively studied. These Gc components appear to be involved in adherence of bacteria to host epithelial cells, in determining serum resistance of Gc, and in inter-Gc association that probably relates to abilities of the organisms to colonize host mucosal surfaces, respectively. Surface exposures of protein I subunits from serum-sensitive versus serum-resistant Gc were shown to differ markedly; P.I subunits of the P.IB type (serum sensitive) display their highly immunogenic central portions on the Gc exterior while serum-resistant Gc's P.IA subunits have only a terminal, poorly immunogenic portion exposed to the organisms' external environment (Swanson). Studies on pili have concentrated on the genetic control mechanisms responsible for gain/loss of piliation and have utilized molecularly-cloned Gc DNA containing a pilus subunit (pilin)-encoding gene to probe the Gc genome; the results suggest that two different mechanisms operate in changing piliation phenotype and one of these involves rearrangement of the organism's chromosomal DNA (Swanson). From partial amino acid sequence data, mixed oligonucleotide probes for detection of protein II structural genes were synthesized. These DNA probes were successfully used to define Gc DNA representing protein II structural genetic material (Mayer). Preliminary data suggest that DNA rearrangement involving these DNA sequences probably coincides with changes in expression of the protein II⁺ phenotype.

Studies on the lipopolysaccharides (LPSs) of gonococci revealed marked heterogeneity in their core structures when derived from different strains (but not for variants of a given strain); these structural differences correlate partially with immunochemical distinctions demonstrable with both polyclonal and monoclonal

reagents. Additional findings include demonstration of LPS-protein I interactions which are resistant to rigorous denaturing and solubilization conditions (100°C, in sodium dodecyl sulfate), and these may prove problematic in constructing vaccines based on purified outer membrane proteins and devoid of toxic LPS material (Hitchcock).

Chlamydiae: The complex interactions occurring between host epithelial cells and chlamydiae as well as those involving chlamydiae and the host's immune system are likely mediated by surface-exposed components of these obligate intracellular bacterial pathogens. Two quantitatively dominant outer membrane moieties of chlamydiae, namely major outer membrane protein (MOMP) and lipopolysaccharide (LPS), have received considerable attention. The presence of LPS in the chlamydial outer membrane was clearly established for the first time (Caldwell and Hitchcock) through a combination of monoclonal antibody and biochemical studies. Unlike most bacterial LPSs, the LPS of chlamydiae appears nontoxic to chick embryos perhaps relating to the ability/necessity of chlamydiae to multiply within endophagocytic vacuoles of host cells. Surprisingly, LPS appears to enjoy little, if any, surface exposure on chlamydial elementary bodies. The importance of MOMP as a major immunogen of chlamydiae was extended by finding serotypic determiners or epitopes on MOMP; this determiner is immunoaccessible on the chlamydial surface as deduced from several findings including neutralization of chlamydial infectivity by monoclonal antibodies directed against the MOMP serotype-specific epitope. Primary amino acid sequence was determined for one portion of the MOMP subunit molecule (Barstad) and oligonucleotide probes were constructed against that sequence (Mayer). These probes were used to molecularly clone a portion of the MOMP structural gene into Escherichia coli (Nano). A provocative finding was demonstration that two minor polypeptides (39K and 19K) of the chlamydial outer membrane were present on elementary bodies (infectious form) but not reticulate bodies, and that these polypeptides bound components of the host-cell plasmalemma; these data suggest key roles for these polypeptides in attachment and/or uptake processes of chlamydiae by eucaryotic cells (Hackstadt). Additional data on outer membrane components and the chlamydial infectious cycle included demonstration that proteolytic removal of MOMP and many other membrane proteins had no influence on the organism's ability to attach to, be taken up by, and multiply within tissue culture cells; the 39K and 19K polypeptides mentioned above were resistant to proteolysis, in situ (Hackstadt).

Spotted fever rickettsiae: Generation and use of monoclonal antibodies directed against Rickettsia rickettsii during the past year provided two interesting findings. Monoclonal reagents specific for 170K and 133K moieties demonstrated protective activity when assayed in an acute lethality model in mice (Anacker). Although this model does not strictly parallel naturally-occurring infections by this rickettsial agent, the findings suggest that antibodies directed toward the same epitopes may yield protective behavior against infections by these intracellular parasites so the target antigens might be vaccine candidates. The second utility of recently-developed monoclonal antibodies is in identification of virulent spotted fever group rickettsiae in hemolymph of ticks whose infected status is questioned when they recovered from patient or other sources. One monoclonal is being used for this purpose since preliminary studies show that it reacts with virulent strains of R. rickettsii but not with some avirulent ones (Anacker and Burgdorfer).

Spirochetes of relapsing fever and Lyme disease: The serotype antigen of Borrelia hermsii (relapsing fever) is outer membrane protein I (pI) that exists in a multitude of subunit forms among relapse isolates from a given strain (Barbour). Purification of pIs from several different serotypes (Barstad) allowed partial amino acid sequence determination on which mixed oligonucleotide probes were constructed (Mayer). These probes are being used both to molecularly clone the pI structural genes from Borrelia genomic DNA and to investigate the mechanisms by which these organisms sequentially express one, then another surface antigen as relapse infections occur (Barbour).

Much less variation is found in surface antigens of the Lyme disease-causing spirochetes which appear to be related to other Borrelia spp. Two outer membrane proteins (31K and 34K) stimulate antibody reactivities in patients with Lyme disease. The 31K is invariant among diverse strains, whereas reactivities of 34K proteins differ among strains as defined by monoclonal reagents; four or more antigenic varieties of the 34K protein have been found to date. An additional antigen (41K) is recognized by antibodies in Lyme disease patients' sera (Barbour). The 31K, 34K, and 41K proteins' structural genes from the Lyme disease spirochete's genomic DNA have been molecularly cloned into E. coli. These recombinant plasmids express their respective products in E. coli, and these are reactive with appropriate monoclonal antibodies. The 31K and 34K polypeptides are encoded on a 6kb fragment of DNA from the Lyme disease spirochete (Howe). Investigations on this agent have been greatly accelerated by ability to propagate the organism in relatively large quantities in vitro (Barbour).

Q fever: Coxiella burnetii, the causative agent of Q fever, has been found to display smooth versus rough LPSs in phase I (virulent) versus phase II (avirulent), and an intermediate "semi-smooth" LPS was identified in C. burnetii isolated from guinea pig placenta nearly a year after original infection with phase I organisms (Hackstadt). Immunochemical and biochemical characteristics of these different LPS forms are being investigated. The three different LPS forms of C. burnetii all appear to be nontoxic to chick embryos (at least 1,000-fold less toxic than salmonella LPSs) even though they reveal reactivity with antibodies raised against lipid A (Hitchcock). The relative nontoxicity of LPS of two intracellular pathogens, Chlamydia and C. burnetii, is a fascinating finding which will be extended (e.g., R. rickettsii) to define probable correlations with intracellular growth.

Lipopolysaccharides: Relationships between chemical structure and toxicity of LPS are being investigated for a number of diverse pathogens including salmonellae and gonococci. Of interest is the finding that smooth versus rough salmonella LPSs are the same regarding their LD₅₀s in chick embryos as are LPSs from gonococci of different strains of several intrastain variants; these and additional data question the putative role of lipid A as the toxic moiety of gram-negative bacterial LPSs (Hitchcock). These and studies with LPSs from less well-defined pathogens such as the contagious equine metritis organism and of Vibrio cholerae have been greatly accelerated by recent development of proteinase K digestion, polyacrylamide gel electrophoresis, and silver staining methods that are efficient, informative ways to characterize LPSs short of formal isolation of these molecules (Hitchcock).

Annual Report
Laboratory of Microbial Structure and Function
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1983, to September 30, 1984

ADMINISTRATIVE REPORT

Personnel changes during the past year include arrival of five staff fellows: Tim Howe, Ph.D., Oregon Health Sciences University, Portland, OR; Francis Nano, Ph.D. University of Illinois, Urbana, IL; Gregory McDonald, Ph.D., University of Virginia, Charlottesville, VA; Abbie Moos, V.M.D., University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA; and Nancy Watkins, Ph.D., University of South Carolina, Columbia, SC, and three visiting fellows: Sven Bergstrom, Umea University, Umea, Sweden; Wolfgang Strittmatter, Max-Planck-Institut fur Immunbiologie, Freiburg-Zahringen, West Germany; and You-Xun Zhang, Beijing Institute of Ophthalmology, Beijing, China. Sohair Sabet, on sabbatical leave from Eastern Virginia Medical School, Norfolk, VA; Joe Meier, California Institute of Technology, Pasadena, CA; Karim Hechemy, New York State Department of Health, Albany, NY; and Theresa Joseph, graduate student, University of Montana, Missoula, MT, joined LMSF as Guest Workers. Summer students included Frank LaQuier, (St. Cloud State University), Timothy McInnis and John Kalbfleisch (Carroll College), Lisa Milch (Cornell University), and Calle Gonzales (University of Washington). Several scientists visited LMSF and presented seminars: Lance Perryman, Washington State University, Pullman, WA; Richard Ogden, Agouron Institute, La Jolla, CA; John Boothroyd, Stanford University, Stanford, CA; Thomas Parr, University of Calgary, Calgary, Alberta, Canada; Warren Strober, LCI, NIAID, Bethesda, MD; Harry Smith, University of Birmingham, Birmingham, England; Frederick Ausubel, Harvard University, Cambridge, MA; Robert Kearns, Samuel Roberts Noble Foundation, Ardmore, OK; David Low, Stanford University, Stanford, CA; John Elliott, University of Vermont, Burlington, VA; Richard Moore, University of British Columbia, Vancouver, B.C., Canada; Scott Halperin, University of Minnesota, Minneapolis, MN; Neil Barg, University of Michigan Medical Center, Ann Arbor, MI; and Mel Simon, California Institute of Technology, Pasadena, CA. The Workshop on the Molecular Aspects of Spirochetal Research, organized by Alan Barbour, was held at the Rocky Mountain Laboratories August 13 and 14 with 45 participants and discussants from the United States and The Netherlands.

Annual Report
Laboratory of Microbial Structure and Function
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1983, to September 30, 1984

HONORS AND AWARDS

Journal Editorial Boards:

J. Swanson - Infection and Immunity

Manuscripts from Annual Review of Microbiology, Biochimica et Biophysica Acta, Canadian Journal of Microbiology, Infection and Immunity, Journal of Bacteriology, Journal of Immunology, Journal of Infectious Diseases, Proceedings of the National Academy of Sciences of the United States of America, Science and Sexually Transmitted Diseases were also reviewed by members of LMSF staff.

Professional Posts:

- J. Swanson - Member, Microbiology and Infectious Diseases Research Committee, NIAID, Bethesda, MD
Member, Promotions and Tenure Committee, NIAID, Bethesda, MD
- R. Anacker - Chairman, Nominating Committee, American Society for Rickettsiology and Rickettsial Diseases, Arlie, VA
- A. Barbour - Member, Merit Review Board on Infectious Diseases, Veterans Administration, Washington, DC
Convener, NIH Workshop on Molecular Aspects of Spirochetal Research, Rocky Mountain Laboratories, Hamilton, MT
- H. Caldwell - Faculty Affiliate, Department of Microbiology, University of Montana, Missoula, MT
Convener, Chlamydial Symposium at Annual Meeting of American Society for Microbiology, St. Louis, MO
- T. Hackstadt - Consultant Member, Food and Drug Administration, Orphan Products Development Initial Review Group, Rockville, MD
- L. Mayer - Faculty Affiliate, Department of Microbiology, University of Montana, Missoula, MT
- Visiting Associate Professor, Department of Chemistry, University of Montana, Missoula, MT

Invited Lectures and Participation in Meetings and Symposia:

- J. Swanson - University of Alberta-University of Calgary Conference on Infectious Disease, Invermere, B.C., Canada
Stanford University, Stanford, CA
Genome Rearrangement, UCLA Symposia on Molecular and Cellular Biology, Steamboat Springs, CO

- R. Anacker - University of Montana, Missoula, MT
Workshop at the Annual Meeting of the American Society for
Rickettsiology and Rickettsial Diseases, Arlie, VA
- A. Barbour - Genome Rearrangement, UCLA Symposia on Molecular and Cellular
Biology, Steamboat Springs, CO
University of Minnesota, Minneapolis, MN
Lyme Disease Symposium, Yale University, New Haven, CT
Sixth Annual Symposium on the Biological Implications of
Pathogenicity, Banff, Alberta, Canada
Grosse Ledder Symposium VII, Pathogenesis of Bacterial Infection,
Schloss Gracht, West Germany
University of Vienna, Vienna, Austria
University of Munich, Munich, West Germany
- P. Barstad - Workshop on the Molecular Aspects of Spirochetal Research,
Hamilton, MT
- H. Caldwell - NIH-sponsored Workshop on the Development of Chlamydial Vaccine,
Bethesda, MD
Rocky Mountain Branch of American Society for Microbiology,
Pingree Park, CO
Sixth Annual Symposium on the Biological Implications of
Pathogenicity, Banff, Alberta, Canada
- T. Hackstadt - Chlamydial Symposium, Annual Meeting of American Society
for Microbiology, St. Louis, MO
- P. Hitchcock - University of Alberta-University of Calgary Conference on
Infectious Disease, Invermere, B.C., Canada
- T. Howe - Workshop on the Molecular Aspects of Spirochetal Research,
Hamilton, MT

Other Activities:

- J. Swanson - Reviewed research grants for National Science Foundation,
Washington, DC, and Medical Research Council of Canada,
Ottawa, Canada
- H. Caldwell - Reviewed research grants for National Science Foundation,
Washington, DC, and British Columbia Health Care Research
Foundation, British Columbia, Canada

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00065-11 LMSF

PERIOD COVERED

October 1, 1983, to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Antigens and Classification of Rickettsiae

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---------|---------------|--------------------------|-------------|
| PI: | R. L. Anacker | Research Microbiologist | LMSF, NIAID |
| Others: | L. A. Thomas | Research Microbiologist | EB, NIAID |
| | S. F. Hayes | Bio. Lab. Tech. (Micro.) | ROMB, NIAID |

COOPERATING UNITS (if any)

Dr. K. E. Hechemy, New York State Dept. of Health, Albany, NY

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:

2.4

PROFESSIONAL:

0.8

OTHER:

1.6

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objectives of this project are the determination of the nature and biological properties of rickettsial antigens and constituents and the development of procedures for classification of spotted fever group rickettsiae. Hybridomas producing monoclonal antibodies were prepared from mice infected with Rickettsia rickettsii. Of the 31 monoclonal antibodies thus far tested for immunoglobulin subclass, 11 belonged to the IgG2A subclass, nine to the IgG2B subclass, and seven to the IgG3 subclass. Four did not react with any of the isotyping sera. Immunoblotting tests showed that five of the antibodies complexed with epitopes present on molecules of various sizes; these molecules may be polysaccharide in part since the ability of the determinants to combine with antibody was not affected by treatment with the proteolytic enzyme proteinase K. With "standard" radioimmune precipitation tests, it was found that 20 monoclonal antibodies reacted with a 170,000-dalton antigen, and six monoclonal antibodies precipitated both 133,000- and 32,000-dalton molecules. Only those antibodies specific for the 170,000- and 133,000-dalton antigens protected mice from a lethal challenge with R. rickettsii. Antibodies to the latter antigens were detected in mouse-protective sera from human patients convalescing from Rocky Mountain spotted fever. Immunoelectron microscopy revealed that both protective and unprotective monoclonal antibodies reacted with antigens on the rickettsial surface. An attempt will be made to purify and characterize the 133,000- and 170,000-dalton antigens.

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 AI 00182-05 LMSF

PERIOD COVERED

October 1, 1983, to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Biochemical and Genetical Mechanisms of Obligate Intracellular Parasitism

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: J. C. Williams

Scientist

LMSF, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

PROFESSIONAL

OTHER

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

Transferred to Office of Scientific Director

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00183-05 LMSF

PERIOD COVERED

October 1, 1983, to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Structure and Function of Bacterial Antigens in the Immune Response

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: J. C. Williams

Scientist

LMSF, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:

PROFESSIONAL

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided)

Transferred to Office of Scientific Director

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00193-05 LMSF

PERIOD COVERED

October 1, 1983, to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Gonococcal Surface Components: Structure and Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: J. Swanson

Chief

LMSF, NIAID

COOPERATING UNITS (if any)

Michael Koomey, Stanford University, Stanford, CA

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

1.3

PROFESSIONAL

0.8

OTHER

0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Implication of pili as virulence factors for gonococci (Gc) in causation of acute gonorrhea has led to interest in genetic control of pilus expression and of subunit variation among pilated Gc derived from a single strain. "On-off" control of piliation has been examined by blot (Southern) hybridization of endonuclease-cleaved Gc genomic DNA with probes containing pilin-encoding information in DNA that was molecularly cloned from pilus₊ Gc. Evidence for two different genetic mechanisms relating to pilus₊ \rightleftharpoons pilus₋ phenotype changes is the present interpretation of the findings: some, but not all, pilus₊ \rightarrow pilus₋ transitions (strain MS11) coincide with recombinational events in Gc genomic DNA; only those pilus₊ to pilus₋ changes that accompany no discernible change in blot hybridization patterns yield pilus₊ progeny that can subsequently undergo pilus₊ \rightarrow pilus₋ transitions. These data are interpreted as reflecting (1) recombination/deletion events in pilus structural gene DNA, and (2) genetic control based on nonpilin-structural-gene portion(s) of the Gc pilin/pilus operon. These studies are also aimed at defining the genetic mechanism(s) involved in the high frequency changes in pilin subunits which are found among variants of a given strain and which accompany changes in both antigenicities and host cell-adherence properties of pili.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00194-05 LMSF

PERIOD COVERED

October 1, 1983, to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Genetics of Neisseria gonorrhoeae

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: L. W. Mayer

Expert (Microbiology)

LMSF, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:

2.0

PROFESSIONAL:

1.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The emphasis of this project is to use molecular genetics, including recombinant DNA techniques, to understand the pathogenesis of gonococcal infections and some other infectious diseases of bacterial etiology. The major area of study involves outer surface components, especially outer membrane proteins, and the genes which encode them. Since the best method to study the primary structure of a protein is to predict its amino acid sequence from the sequence of the gene, we have concentrated on gene cloning as the first step. The control of expression is also an interesting area of study which can be pursued by analysis of the cloned DNA segment including the gene(s) of interest. We have used oligonucleotide probes to identify clones of genes encoding outer membrane proteins. A clone of gonococcal PII has been partially characterized. Using this cloned DNA as a probe in Southern blots, we have shown some DNA sequence alteration (probably rearrangement) correlated with phenotypes expressing or not expressing the PII proteins. The control of expression and apparent arrangement of the PII genes seems to have some interesting differences and similarities to the pattern seen for genes of another virulence factor--gonococcal pili. We have also synthesized oligonucleotides for several other laboratories within NIAID for use as linkers, DNA sequencing primers, directed mutagenesis of cloned genes, and probes of cloned genes analogous to our own use of them.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00196-04 LMSF

PERIOD COVERED

October 1, 1983, to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Bordetella pertussis: Outer Membrane Structure and Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: M. S. Peppler

Expert (Microbiology)

LMSF, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

PROFESSIONAL:

OTHER

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type Do not exceed the space provided.)

M. S. Peppler transferred to University of Alberta, Edmonton, Alberta, Canada, in August 1983, therefore this project is terminated.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00216-04 LMSF

PERIOD COVERED

October 1, 1983, to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunochemistry of Chlamydial Surface Antigens

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: H. D. Caldwell Expert (Microbiology) LMSF, NIAID

Others: F. E. Nano Staff Fellow LMSF, NIAID

P. J. Hitchcock Senior Staff Fellow LMSF, NIAID

W. J. Todd Senior Staff Fellow RMOB, NIAID

S. F. Sabet Guest Worker LMSF, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:

1.7

PROFESSIONAL:

0.7

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objective of this project is to identify immunoaccessible surface antigens of Chlamydia trachomatis organisms that are potential candidate antigens for the development of a chlamydial vaccine. The study has focused on two macromolecules, the major outer membrane protein (MOMP) and lipopolysaccharide (LPS). Monoclonal antibodies have been raised against each of these macromolecules. Antibodies (IgG3) that recognized type-, subspecies-, and species-specific epitopes located on MOMP and a genus-specific epitope located on LPS have been identified by immunoblotting analyses and indirect fluorescent antibody. The immunoaccessibility of these antigenic determinants on viable chlamydiae and their biological significance have been studied by the following methods: (i) binding of radioiodinated antibodies by viable organisms, (ii) immunoelectron cytochemistry with protein A colloidal gold as a probe, and (iii) neutralization of in vitro infectivity. The findings show that only the type-specific antigenic determinant is immunoaccessible on viable chlamydiae. Epidemiological data indicate that either immunization or natural infection confers partial immunity, and this immunity is restricted to the infecting C. trachomatis serotype. These observations suggest that the peptide fragment of MOMP which contains the type-specific determinant would be a reasonable candidate for a subunit peptide vaccine for C. trachomatis infections. A 15Kd peptide with these antigenic properties has been identified from CNBr digests of MOMP (Project Z01 AI 00233-03 LMSF) and the nucleotide sequence of the MOMP gene that codes for this determinant has been cloned in Escherichia coli (Z01 AI 00413-01 LMSF). The function of MOMP and LPS in chlamydial attachment, penetration, and inhibition of phagolysosome fusion is also being studied in collaboration with Dr. Ted Hackstadt and Dr. Sue F. Sabet (Guest Worker).

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00230-03 LMSF

PERIOD COVERED

October 1, 1983, to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Virulence-Associated Factors of *Rickettsia rickettsii*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R. L. Anacker

Research Microbiologist

LMSF, NIAID

Others: W. Burgdorfer

Res. Entomologist (Medical) EB, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:

0.6

PROFESSIONAL:

0.2

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are attempting to identify and characterize those factors of *Rickettsia rickettsii* which enable the rickettsiae to invade and grow in mammalian cells. In the past year we have found a monoclonal antibody which, in all the screening tests we have done thus far, reacts in the immunofluorescence test with virulent *R. rickettsii* but not with other avirulent and virulent rickettsiae of the spotted fever group. This monoclonal antibody reacted with five hemolymph-associated organisms of the 34 microbial agents found in a survey of 310 ticks collected in the Bitter Root Valley of western Montana. These results offer hope that this monoclonal antibody may be used to identify individuals bitten by *R. rickettsii*-parasitized ticks well before symptoms of the disease would be apparent. In a separate study, two substances, presumably located on the surface of *R. rickettsii*, were found to preferentially adsorb to glutaraldehyde-fixed L cells. As yet, we have not determined whether these factors are actually involved in the infection of susceptible cells by viable rickettsiae.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00231-03 LMSF

PERIOD COVERED

October 1, 1983, to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biology of Relapsing Fever Borrelia spp.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: A. G. Barbour Senior Staff Fellow LMSF, NIAID

Other: J. Meier Guest Worker LMSF, NIAID

COOPERATING UNITS (if any)

Dr. Melvin Simon, California Institute of Technology, Pasadena, CA

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:

1.35

PROFESSIONAL:

0.5

OTHER:

0.85

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The relapsing fever spirochete, Borrelia hermsii, demonstrates a clonal polymorphism of its major surface antigens, the pI proteins. Differential expression of one of several distinct pI proteins appears to be the basis for the antigenic variation that occurs during relapsing fever. Our current studies are focused on the molecular mechanism of antigen switching in borreliæ, and to this end, we are isolating the gene or genes for pI proteins. This project has been aided by the development of several monoclonal antibodies that are either serotype-specific or cross-reactive between serotypes and by partial amino acid sequencing of two pI proteins. These studies should not only lead to a better understanding of the pathogenesis of an infectious disease that has had during its epidemics historical impacts, but may also reveal more about gene expression in procaryotic organisms.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00232-03 LMSF

PERIOD COVERED

October 1, 1983, to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pathogenesis of Lyme Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: A. G. Barbour

Senior Staff Fellow

LMSF, NIAID

COOPERATING UNITS (if any)

Dr. Gerold Stanek, Institute of Hygiene, University of Vienna, Vienna, Austria

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

1.35

PROFESSIONAL

0.4

OTHER

0.95

CHECK APPROPRIATE BOX(ES)



(a) Human subjects



(b) Human tissues



(c) Neither

☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Spirochetes that were originally isolated from Ixodes spp. ticks are the etiologic agents of Lyme disease, erythema chronicum migrans and tick-associated chronic meningitis. Our studies of the pathogenesis of Lyme disease and related disorders have focused on proteins that are immunogenic during human infection and the surface components of the spirochete. We have to date identified in the Lyme disease spirochetes three major antigens, two of which are abundant surface proteins. The genes for these two surface proteins have been cloned into Escherichia coli. Monoclonal antibodies specific for each of the three antigens were developed. Monoclonal antibodies against one of the surface proteins differed in their reactivities with different isolates. There appear to be at least four distinct serotypes among the strains examined. These studies and the reagents we have developed have provided some understanding of the antigenic "make-up" of these spirochetes and will provide the basis for further investigations on the pathogenesis of these acute and chronic bacterial diseases.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00233-03 LMSF

PERIOD COVERED

October 1, 1983, to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structural Analysis of the Major Outer Membrane Protein of Chlamydia trachomatis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: H. D. Caldwell

Expert (Microbiology)

LMSF, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

1.3

PROFESSIONAL:

0.3

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objective of this project is to identify peptide fragment(s) of the Chlamydia trachomatis major outer membrane protein (MOMP) that possess type-specific antigenic properties. This antigenic determinant is highly immunoaccessible on viable chlamydiae and has been identified as a potential peptide candidate for the development of a C. trachomatis vaccine (Project Z01 AI 00216-04 LMSF). Peptide fragments of MOMP were generated by Cyanogen bromide (CNBr) cleavage and Staphylococcus aureus V8 protease digestion. The antigenic properties of peptides were analyzed by immunoblotting with monoclonal antibodies that recognize type-, subspecies-, and species-specific epitopes located on the same MOMP polypeptide. Limited proteolysis of MOMP with V8 protease resulted in nine immunoreactive peptides ranging in molecular weight from 7Kd to 25Kd when probed with polyclonal anti-MOMP IgG. Two of these fragments (13Kd and 18Kd) reacted with the subspecies- and species-specific MOMP monoclonal antibodies, indicating that these epitopes are closely associated on the intact MOMP polypeptide. The type-specific monoclonal antibody, although reactive with undigested MOMP, did not react with any of the V8 protease MOMP peptide fragments. These findings suggest that the type-specific MOMP antigenic determinant contains or is in close proximity to a glutamic acid residue. CNBr digestion of MOMP resulted in immunoreactive peptides of 21Kd, 15Kd, and 9Kd with polyclonal anti-MOMP IgG. The subspecies- and species-specific MOMP monoclonal antibodies reacted with the 21Kd and 9Kd fragments, whereas the type-specific MOMP monoclonal antibody recognized only the 15Kd peptide. The isolated 15Kd fragment will be used as an immunogen to determine its potential as a subunit or peptidic C. trachomatis vaccine.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00234-03 LMSF

PERIOD COVERED

October 1, 1983, to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Biology of Intracellular Parasitism

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: T. Hackstadt Senior Staff Fellow LMSF, NIAID

Others: H. D. Caldwell Expert (Microbiology) LMSF, NIAID
 M. G. Peacock Research Microbiologist EB, NIAID

COOPERATING UNITS (if any)

L. P. Mallavia, Dept. Bacteriology and Public Health, Washington State University
 M. S. Peppler, Dept. Medical Microbiology, University of Alberta

LAB/BRANCH

Laboratory of Microbial Structure and Function

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

3.1

PROFESSIONAL:

1.1

OTHER

2.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project examines mechanisms of pathogenesis of diverse groups of obligately intracellular bacteria. Representative members of different genera with varied sites of replication in the host cell are being examined. Coxiella burnetii, the etiologic agent of Q fever replicates within the normally bactericidal phagolysosome. Chlamydiae, in contrast, block phagosome-lysosome fusion and in doing so prevent their destruction and clearance from the host cell. Current interest centers on comparative aspects of intracellular survival of these two agents. Initial events of chlamydia-host interaction including attachment, internalization, inhibition of phagosome-lysosome fusion, and differentiation are being studied with an emphasis on the role of disulfide mediated interactions and exchange in the chlamydial life cycle. In addition, we have identified two chlamydial proteins, present on the infectious stage of the chlamydial life cycle but absent from the noninfectious form, that bind host cell surface protein thus may potentially be involved in regulating host response to chlamydial infection. A different approach has been taken in the study of C. burnetii. This rickettsia undergoes a virulent to avirulent transition termed phase variation. The only components identified as structurally and antigenically unique between phases were the lipopolysaccharides (LPS). As part of this investigation, we have identified a previously unknown third LPS type intermediate in structure between the virulent and avirulent LPS. The organism possessing this unusual LPS was isolated from the placental tissue of a guinea pig infected almost a year previously with C. burnetii. Possible roles of this LPS variation in the known capacity of C. burnetii for persistent or chronic infection of humans and animals are being examined.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00235-03 LMSF

PERIOD COVERED

October 1, 1983, to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on the Structure and Function of LPSs of Pathogenic Bacteria

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: P. J. Hitchcock Senior Staff Fellow LMSF, NIAID

Other: H. D. Caldwell Expert (Microbiology) LMSF, NIAID

T. Hackstadt Senior Staff Fellow LMSF, NIAID

W. J. Todd Senior Staff Fellow RMOB, NIAID

T. M. Brown Microbiologist LMSF, NIAID

COOPERATING UNITS (if any)

Dr. David C. Morrison, Department of Microbiology, Emory University School of Medicine, Atlanta, GA,

Dr. Linda Shoer, List Biological Laboratories, Inc., Campbell, CA

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

1.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Lipopolysaccharide (LPS) comprises about 40% of the gram-negative outer membrane (OM). Due to its biochemical composition and surface location, LPS mediates a number of immune and nonimmune host responses. Analysis of the structure and function is essential for understanding the role of LPS in the pathogenesis of disease. This is a multifocal project with two basic themes: (A) Development of reagents and techniques for use in evaluating bacterial LPSs, and (B) Analysis and comparison of LPSs of pathogenic bacteria. Specific aspects of this project include first, development and analysis of SDS-PAGE as a tool for fingerprinting LPSs, this necessitates identification of the catalog of artifacts influencing the behavior of LPS migration: pH as well as SDS concentration are critical components in mediation of aberrant LPS migration. Analysis of SDS-PAGE/immuno-electroblotting techniques for probing the antigenicity of LPS demonstrates steric factors markedly affect epitopes available for antibody binding. Second, analysis of the chick embryo model as a bioassay for LPSs, the chick embryo assay is unique in that the toxic principle is not lipid A. The LPSs of two intracellular pathogens are nontoxic in this bioassay. Third, LPSs of three venereal pathogens are being analyzed and compared. These LPSs are similar in that 0 antigens are absent. The LPS of Neisseria gonorrhoeae is heterogeneous depending upon the strain, no heterogeneity has been detected within a given strain with respect to piliation or opacity phenotype. The LPS of Chlamydia trachomatis L2 (LGV strain) has also been isolated; like the LPS of N. gonorrhoeae, it too cross-reacts with antisera made to Salmonella lipid A and Re LPS. However, the LPS of C. trachomatis is nontoxic in the chick embryo assay. The rough LPS of Contagious Equine Metritis Organism (CEMO) also cross-reacts with antibodies to lipid A but not with antibodies directed against Re LPS. This is consistent with the absence of KDO in the TBA assay. Fourth, the association of LPS with OM protein constituents (protein I and pili) is being examined.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00236-03 LMSF

PERIOD COVERED

October 1, 1983, to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Studies on the Biology of Contagious Equine Metritis Organism

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---------|-----------------|--------------------------|-------------|
| PI: | P. J. Hitchcock | Senior Staff Fellow | LMSF, NIAID |
| Others: | W. J. Todd | Senior Staff Fellow | RMOB, NIAID |
| | T. M. Brown | Microbiologist | LMSF, NIAID |
| | O. Barrera | Microbiologist | LMSF, NIAID |
| | D. Corwin | Bio. Lab. Tech. (Micro.) | RMOB, NIAID |
| | S. F. Hayes | Bio. Lab. Tech. (Micro.) | RMOB, NIAID |

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

1.2

PROFESSIONAL

0.5

OTHER

0.7

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objective of this project is the characterization of a recently defined veterinary pathogen--Contagious Equine Metritis Organism (CEMO). Clinically contagious equine metritis (CEM) shares some of the features of gonorrhea in humans. Specifically leukorrhea, silent infections, chronic infections, and the generation of nonprotective antibodies during the course of infection occur in both diseases. Interest in CEM as a possible animal model for gonorrhea and interest in the mechanisms of pathogenesis prompted these studies. The project is multifaceted; specific aspects include (1) ultrastructural analysis of colonial variants of three strains, (2) analysis and comparison of the major outer membrane protein (MOMP), (3) analysis of strain specific surface proteins, (4) isolation and characterization of capsule and lipopolysaccharide (LPS, see project Z01 AI 00235-03, and (5) production of monoclonal antibodies to surface proteins, capsule and LPS. Like Neisseria gonorrhoeae, organisms grown on clear typing medium express colonial phenotypic variation. Opaque and transparent phenotypes have been isolated, cloned and evaluated. Unlike the gonococcus, the basis for opacity variation does not appear to correlate with protein variation. In addition, several capsule/LPS mutants have been isolated based on a colonial characteristic of intermediate opacity. The organism produces large amounts of extracellular material, capsule. This capsule, likely carbohydrate in composition, is extremely antigenic in experimentally infected horses and hyperimmunized rabbits. The MOMP of CEMO has been evaluated by SDS-PAGE, it has the same M_r as MOMP of N. meningitidis (41K). Two-dimensional peptide maps of MOMP from these two species are remarkably similar. Other surface constituents of interest include several strain specific proteins (these are antigenic) and the LPS (see Z01 AI 000235-03 LMSF). We are producing monoclonal antibodies to CEMO surface constituents and presently have two--one reactive with capsule and one with MOMP.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00238-02 LMSF

PERIOD COVERED

October 1, 1983, to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Bordetella pertussis and Bordetella bronchiseptica: Outer Membrane Structure

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: M. S. Pepler

Expert (Microbiology)

LMSF, NIAID

COOPERATING UNITS (if any)

None

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Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

M. S. Pepler transferred to University of Alberta, Edmonton, Alberta, Canada, in August 1983, therefore this project is terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00239-03 LMSF

PERIOD COVERED

October 1, 1983, to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Gonococcal Surface Proteins' Immunochemical Characteristics

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: J. Swanson

Chief

LMSF, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

0.7

PROFESSIONAL:

0.2

OTHER

0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

During this reporting period, studies on this project have concentrated on defining differences in surface exposure of a particular outer membrane protein--protein I--of gonococci (Gc). This protein's subunit size correlates with the organism's susceptibility to killing by normal human sera and the organism's ability to cause disseminated Gc infection. The findings reveal a marked difference in the manner in which protein I (P.I) of serum-resistant Gc is disposed in the outer membrane in comparison with P.I of serum-sensitive Gc. Attempts are underway to define the surface-exposed portions of these P.I subunits through generation of monoclonal antibodies; development of monoclonal reagents directed against additional outer membrane proteins (P.II, P.III) and against both common and unique portions of pilus-subunit molecules (pilin).

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00362-02 LMSF

PERIOD COVERED

October 1, 1983, to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Primary Structural Analysis of Bacterial Membrane Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: P. A. Barstad

Senior Staff Fellow

LMSF, NIAID

COOPERATING UNITS (if any)

John Coligan, LIG, NIAID

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:

1.7

PROFESSIONAL:

1.0

OTHER:

0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to obtain sufficient amino acid sequence analysis from pathogenic bacterial surface membrane proteins to facilitate isolation of the corresponding genes using synthetic oligonucleotide mixtures. The genes will then be sequenced by dideoxynucleotide or Maxim-Gilbert methods, and some will be evaluated for use in vaccines. Several milligrams of the serotype specific proteins (SSPs) (formerly called PIs) from Borrelia hermsii have been isolated by selective detergent extraction followed by reverse phase HPLC. Both the type 7 and type 21 SSPs have blocked amino termini. Cyanogen bromide cleavage of the SSPs and analysis of the fragments by automated Edman degradation and Western blot analysis indicate (1) each B. hermsii SSP represents a different gene product and (2) there is sufficient regions of sequence homology between these SSPs to indicate that they evolved from a common ancestral gene. These observations suggest that the SSPs in B. hermsii represent the first large bacterial multigene family identified to date and provide an excellent model to study gene activation in bacteria at the molecular level. Clones from Chlamydia trachomatis LGV-2 have been selected using a synthetic oligonucleotide made to residues 12-16 of the major outer membrane protein (MOMP). DNA sequence analysis has generated approximately 1 Kb of information from a 5-Kb fragment, but the nucleotide sequence corresponding to the MOMP amino terminus has not yet been located.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00363-01 LMSF

PERIOD COVERED

October 1, 1983, to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

The Role of Obligate Intracellular Bacteria as the Etiological Agent of AIDS

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: H. D. Caldwell

Expert (Microbiology)

LMSF, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

PROFESSIONAL

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

This project has been terminated.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00364-01 LMSF

PERIOD COVERED

October 1, 1983, to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of Lipopolysaccharide by SDS-Polyacrylamide Gel Electrophoresis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: P. J. Hitchcock

Senior Staff Fellow

LMSF, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been combined with Project No. Z01 AI 00235-03 LMSF.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00412-01 LMSF

PERIOD COVERED

October 30, 1983, to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Molecular Analysis of the Lyme Disease Spirochete

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: A. G. Barbour Senior Staff Fellow LMSF, NIAID

Others: T. R. Howe Staff Fellow LMSF, NIAID
L. W. Mayer Expert (Microbiology) LMSF, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

1.1

PROFESSIONAL:

1.1

OTHER

0.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

The etiological agent of Lyme disease (LD) is a tick-borne, hitherto unknown spirochete of the genus Borrelia (see Projects Z01 AI 00232-03 LMSF and Z01 AI 00268-03 EB). We are studying pathogenesis of LD, and to obtain useful reagents for this study as well as to understand the biology of the etiological agent, we have initiated a molecular approach. To date we have prepared a gene bank of LD spirochete DNA in Escherichia coli and have isolated three recombinant clones expressing LD spirochete antigens. One of the clones produces both of the major, outer-membrane associated proteins of LD spirochete B31. These and other clones will be examined in depth and will be used to assess the feasibility of immuno-prophylaxis of LD.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00413-01 LMSF

PERIOD COVERED

October 16, 1983, to August 12, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Genetics of *Chlamydia trachomatis*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: H. C. Caldwell Expert (Microbiology) LMSF, NIAID

Others: F. E. Nano Staff Fellow LMSF, NIAID

P. A. Barstad Senior Staff Fellow LMSF, NIAID

T. Joseph Graduate Student LMSF, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:

1.3

PROFESSIONAL:

1.1

OTHER:

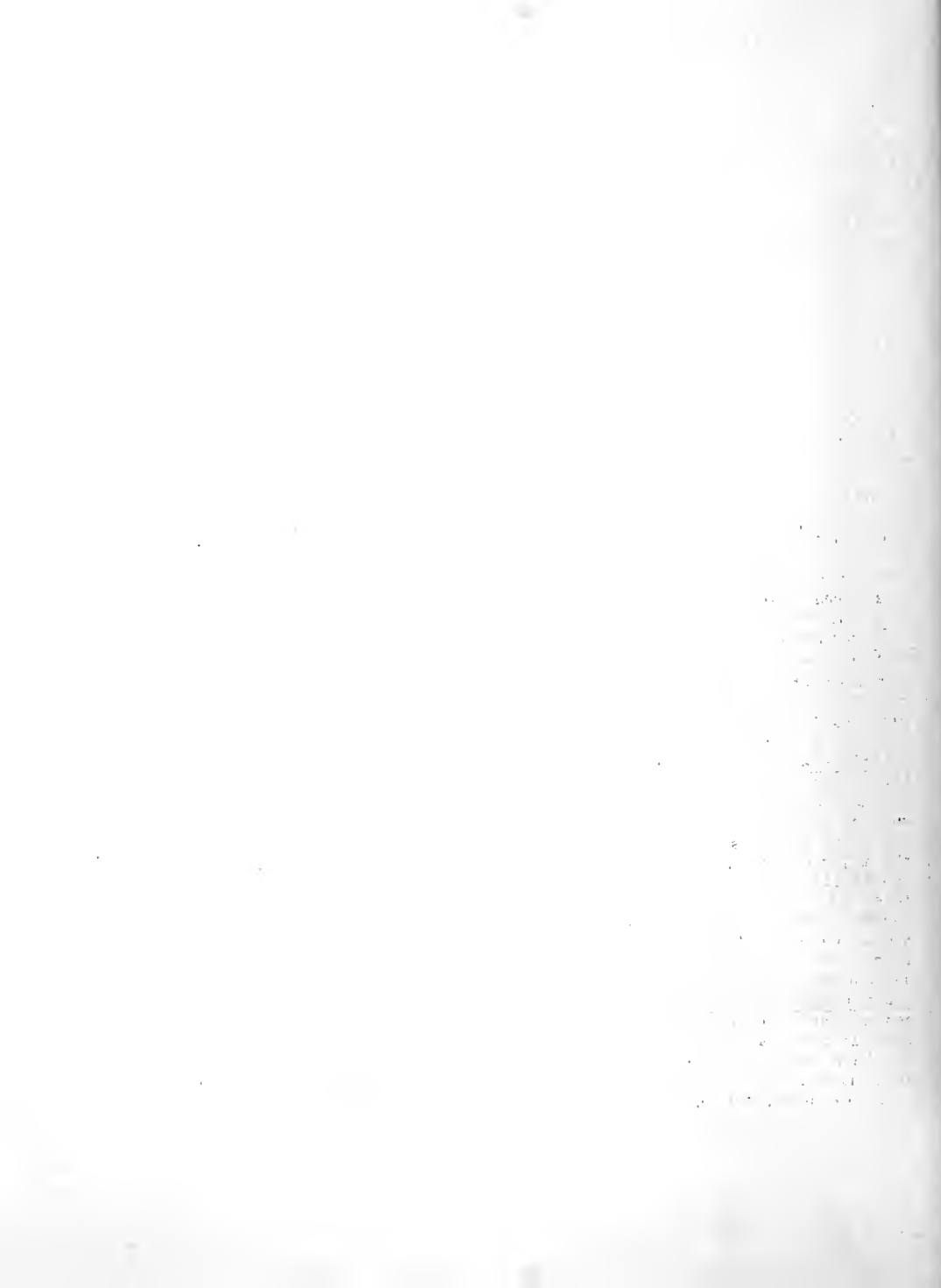
0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A number of molecular genetic approaches are being employed to complement biochemical studies of the pathobiology of *Chlamydia trachomatis*. In collaboration with Harlan Caldwell and others in this laboratory, the following projects are being pursued: (1) Cloning of genes encoding outer membrane components of *C. trachomatis*. A variety of cloning strategies have been used in order to isolate the genes encoding surface components of *C. trachomatis* and *C. psittaci*. It is hoped that a comparison of conserved structural domains between similar surface molecules of *C. trachomatis* and *C. psittaci* will point to functionally important segments of the molecule. From the amino acid sequence of a portion of the major outer membrane protein (MOMP) of *C. trachomatis*, an oligonucleotide was constructed that was predicted to hybridize to the gene encoding the MOMP. Using this oligonucleotide as a DNA probe, we isolated a recombinant clone that produced a 15Kd peptide that reacted with monoclonal antibody directed against the MOMP. We have cloned larger DNA segments in order to achieve expression of the intact 40Kd MOMP but have been unsuccessful with this approach. We are currently sequencing the MOMP gene and surrounding DNA sequences. (2) DNA sequence analysis of chlamydial plasmids. The plasmids of *Chlamydia* are suspected of coding for gene products essential to pathogenesis. As an initial analysis of the plasmids, we have cloned the entire single plasmids from different strains and have begun DNA sequence analysis. Knowledge of the DNA sequence will allow us to manipulate the expression of open reading frame segments of the plasmid and to follow plasmid transcription.





Laboratory of Persistent Viral Diseases
Rocky Mountain Laboratories
Hamilton, Montana
1984 Annual Report
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Project Number

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Annual Report
Laboratory of Persistent Viral Diseases
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1983 to September 30, 1984

RESEARCH HIGHLIGHTS

New focal immunofluorescence assay (FIA) developed to quantitate viruses in live cell monolayers. Using monoclonal antibody probes, this assay has now been used with several classes of viruses which induce expression of viral cell surface proteins, including rabies, herpes simplex and many groups of retroviruses (Sitbon, Chesebro, Lodmell).

pH dependent fusion of Mus dunii cells by retroviruses was determined to be a property encoded by the amino-terminal portion of the envelope gene. This conclusion was reached by comparing retroviruses with recombinations within the envelope gene (Portis, Evans).

Different strains of ecotropic murine leukemia virus were found to recombine with different endogenous viral genes in the generation of dual-tropic viruses (Evans).

Two new strains of recombinant dual-tropic viruses derived from ecotropic Friend murine leukemia virus were capable of inducing leukemia in the absence of parental ecotropic virus. Furthermore sequential inoculation of dual-tropic virus followed by ecotropic virus led to acceleration of appearance of leukemia. This implied the existence of complementary interactions between these viruses, perhaps due to alteration of cellular tropism through phenotypic mixing (Chesebro, Evans).

Rous sarcoma virus (RSV) capable of transforming erythroid cells in vitro and in vivo. Similar to RSV transformation of fibroblasts, the RSV-transformed erythroid cells were blocked in differentiation, and when temperature sensitive mutant viruses were used, the transformed phenotype could be reversed at non-permissive temperatures (Palmieri).

Genetically controlled resistance to rabies virus in mice appears to act via immune response mechanisms. Resistance was transiently abolished by treating mice with cyclophosphamide. Also resistance could be transferred to susceptible mice by adoptive transfer of immune spleen cells (Lodmell).

Virulent Aleutian disease virus strain Utah I structural gene cloned. This clone was similar to a previous clone from the avirulent Gorham strain in that it expressed ADV-specific protein in *E. coli* cells and in an *in vitro* transcription-translation system. No differences in the protein from these strains have been identified so far (Bloom).

Monoclonal antibodies specific for Aleutian disease virus strains indicate different intracellular distribution of viral structural proteins of virulent and avirulent virus strains (Race).

Female protein identified as a component of amyloid in hamsters. The serum female protein pool appears to be in equilibrium with the female protein deposited in amyloid. Thus the amyloid does not constitute a depot which is sequestered away from other catabolic homeostatic processes (Coe).

Murine antibody response to human gamma globulin (HGG) was found to be entirely specific. The increase in spleen IgG₁ secreting cells following immunization correlated exactly with the increase in cells secreting anti-HGG antibody. Thus, there appeared to be no nonspecific component to this immune response (Etlinger).

Annual Report
Laboratory of Persistent Viral Diseases
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1983 to September 30, 1984

ADMINISTRATIVE REPORT

New Staff Fellows arriving at LPVD this year include: Dr. Richard Buller, Ph.D., University of Montana; Dr. John Morrey, Ph.D., Utah State University; Dr. Sheryl Lard, Ph.D., Colorado State University; Dr. Daniel Wiedbrauck, Ph.D., University of Notre Dame. Other personnel changes were the departures of Dr. Miles Cloyd, Senior Staff Fellow, Dr. Anders Cohn, a Guest Worker from Copenhagen, Denmark and Mrs. Margie Thompson, GS-9 technician.

Summer students were: Cyndra Coffing, Barry Grosser, David Lechner, Carroll College, Helena, MT; Mari Vogel, University of Montana, Missoula, MT

Annual Report
Laboratory of Persistent Viral Diseases
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1983 to September 30, 1984

HONORS AND AWARDS

Professional Posts:

- Dr. B. Chesebro - Adjunct Professor - Department of Microbiology,
Montana State University, Bozeman, MT
- Dr. M. E. Bloom - Faculty Affiliate, Department of Microbiology,
University of Montana, Missoula, MT
- Dr. J. E. Coe - Faculty Affiliate, Department of Microbiology,
University of Montana, Missoula, MT
- Dr. D. L. Lodmell - Faculty Affiliate, Department of Microbiology,
University of Montana, Missoula, MT

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00074-12 LPVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetically Controlled Mechanisms of Recovery From Friend Virus-Induced Leukemia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: B. Chesebro Chief LPVD, NIAID

Others: R. Morrison Staff Fellow LPVD, NIAID
M. Sitbon Visiting Fellow LPVD, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

3.0

PROFESSIONAL:

2.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unspaced type. Do not exceed the space provided.)

Recovery from Friend virus (FV)-induced leukemia in mice is influenced by the Rfv-1, Rfv-2 and Rfv-3 genes. The H-2D-linked Rfv-1 gene appears to affect the FV-specific T lymphocyte response kinetics. Current data using monoclonal anti-L3T4 and anti-Ly2 antibodies indicate that the main T cell response detected in vitro from both high and low recovery phenotype mice (H-2D^{b/b} and H-2D^{d/b}) involves helper T lymphocytes, which recognize antigen together with I-A gene products. Influence of the H-2D region on this response has not been noted previously in other systems. In other experiments, mice with the bml4 mutation in the H-2D region were found to have a decreased incidence of recovery from FV leukemia. A new fluorescent immunoassay using monoclonal antibodies to detect foci of viral infection in live cell monolayers has been developed. This assay has facilitated quantitation of retroviruses, rabies virus and herpes simplex virus, as well as permitted rapid biological cloning of viruses in complex mixtures.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00260-03 LPVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders) **Role of Endogenous and Recombinant Retroviruses in Leukemia and Differentiation**

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: B. Chesebro

Chief

LPVD, NIAID

Others: M. Sitbon

Visiting Fellow

LPVD, NIAID

COOPERATING UNITS (if any)

None

LAB BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

2.0

PROFESSIONAL

1.0

OTHER

1.0

CHECK APPROPRIATE BOX(ES)



(a) Human subjects



(b) Human tissues



(c) Neither



(a1) Minors



(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

Inoculation of mice with Friend murine leukemia virus (F-MuLV) induces leukemia in erythroid, myeloid and lymphoid lineages. Our previous data indicated that dual-tropic recombinant Friend MCF (F-MCF) viruses generated in vivo after F-MuLV inoculation were not required for myeloid or lymphoid leukemogenesis. However, in certain mouse strains there was a strong correlation between expression of F-MCF viruses and erythroid leukemia. Possible leukemogenic effects of F-MCF viruses isolated free of F-MuLV were studied by inoculation of newborn mice. Only 2 of 12 isolates were leukemogenic. These two isolates induced mostly erythroid leukemia, though occasional lymphoid and myeloid leukemias were observed. Surprisingly, the latent period was longer with F-MCF viruses than with F-MuLV. Furthermore, inoculation of F-MCF at birth followed by F-MuLV at 6 weeks caused an accelerated appearance of leukemias. The most likely interpretation of these results is that phenotypic mixing of envelopes and RNAs of these two viruses leads to more rapid infection of cell types which are highly susceptible to leukemic transformation.

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 AI 00072-13 LPVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Host and Viral Factors in Resistance to Rabies Virus Infection in Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: D. L. Lodmell

Scientist Director

LPVD, NIAID

Others: None

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:

2.0

PROFESSIONAL:

1.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

The principal objective of these studies is to determine host and viral factors which influence the genetically-controlled resistance of inbred mouse strains to street rabies virus (SRV). It has been shown that the two segregating genes of SJL and CBA/J mice responsible for resistance to SRV were allelic (identical). In contrast, resistance genes of SJL and BALB/c, SJL and DBA/2, CBA/J and BALB/c and CBA/J and DBA/2 were nonallelic (not identical). In these instances there was one different resistance gene for each strain. Cell transfer studies have shown that nonimmune spleen cells of resistant mouse strains did not protect unirradiated H-2 histocompatible susceptible strains against SRV. In contrast, 1×10^7 immune spleen cells of resistant BALB/c (nu/+) mice were highly effective in protecting susceptible BALB/c (nu/nu) mice if cells were inoculated 1-7 days before SRV. Furthermore, susceptible athymic mice which had been infected 3 days previously with SRV, and were known to have spinal cord infections, also were protected by immune cells. Anti-SRV antibody alone did not protect these mice. Additional experiments showed that immune spleen cells harvested from BALB/c (nu/+) mice 3-370 days after SRV infection transferred resistance against SRV. All nu/nu survivors which received immune cells had moderate (1:320) to high (1:5120) serum neutralizing antibody titers. It also was determined that cyclophosphamide abolished the resistance of SJL mice to SRV. Prior to their death, none of these mice was able to produce neutralizing antibody to SRV. In contrast, SJL mice inoculated with SRV after the immunosuppressive effects of cyclophosphamide had abated produced neutralizing antibody and survived. Additional experiments showed that passively transferred immune cells or immune serum protected SJL mice which were unable to produce neutralizing antibody against SRV following cyclophosphamide treatment. Direct infection of the CNS of SJL mice via intranasal or intracerebral challenge determined that serum anti-SRV neutralizing antibody was essential to genetic resistance as well as CNS immunity. Similar antibody within the CSF was not associated with resistance to SRV.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00261-03 LPVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunological Aspects of Neurovirology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: D. L. Lodmell

Scientist Director

LPVD, NIAID

Others: None

COOPERATING UNITS (if any)

None

LAB BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

2.0

PROFESSIONAL

1.0

OTHER

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The principal objective of these studies is to delineate the significance of immune responses within the central nervous system (CNS) of mouse strains which vary in their resistance to the neurotropic street rabies virus (SRV). Anti-SRV neutralizing antibody was not detected in the cerebrospinal fluid (CSF) of resistant or susceptible mice for the first 10 days postinoculation. It was initially detected in the highly resistant-asymptomatic SJL and CBA mice after the 15th day. Neutralizing antibody was not detected in the CSF of mice with serum neutralizing antibody titers of less than 1:320. Nonetheless, anti-SRV neutralizing antibody in the CSF might have been important in resolving infections of mice which survived following development of clinical CNS disease. It appeared in high titer in both DBA/2 and BALB/c strains during their recuperation period (>10 days postinoculation), and was not present in sick susceptible A.SW/Sn mice which were alive 12 days after SRV inoculation, but were destined to die. Direct infection of the CNS via intranasal (IN) challenge has shown that anti-SRV neutralizing antibody in the CSF is inconsequential in genetic resistance or CNS immunity to SRV. In contrast, serum neutralizing antibody was essential in both instances since SJL mice with this antibody, but without CSF antibody (5-7 days postinoculation) did not die when challenged IN. In contrast, mice with neither serum nor CSF antibody (1-3 days postinoculation) died. Studies with nude mice, which make a minimal neutralizing antibody response to SRV, have shown that SRV was not present in the CSF of mice which had brain and spinal cord virus titers exceeding $10^{5.0}$ MICLD₅₀/0.03 ml.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00073-19 LPVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms of Immunity and Immunopathology Related to Cellular/Humoral Immunity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J. E. Coe

Medical Officer

LPVD, NIAID

Others: None

COOPERATING UNITS (if any)

Dr. K. Ishak, AFIP, Washington, DC; Dr. R. F. Schell, Division Infect. Dis., Hahnemann Medical College, Philadelphia, PA; Dr. S. Reed, Cornell Medical Center, New York, NY; Dr. C. Kirk Phares, Univ. Nebraska Medical Center, Omaha, NB

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NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:

0.2

PROFESSIONAL:

0.1

OTHER:

0.1

CHECK APPROPRIATE BOXES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Syrian hamster is a suitable host for several infectious diseases. This laboratory is studying the inflammatory and immune response in various hamster infectious disease models which are ongoing in other investigators' laboratories. We are interested in any involvement (helpful or injurious) of acute phase reactants (female protein) and also wish to determine the isotype of antibody and correlate these factors with the resultant immunopathology. In a persistent treponema model, hamsters have been found to become infected with syphilis and yaws and protective immune immunoglobulins have been isolated and identified. Two parasitic models also are being studied, 1) Leishmania produces a chronic disease in hamsters similar to human infection and an unusual deposit of amyloid in glomerulae of female hamsters, and 2) Plerocercoid infected hamsters also develop extensive amyloid, even in males which have increased serum FP levels.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00262-03 LPVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Pentraxins in Acute and Chronic Pathology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: J. E. Coe

Medical Officer

LPVD, NIAID

Others: H. Etlinger

Senior Staff Fellow

LPVD, NIAID

COOPERATING UNITS (if any)

Dr. H. Gewurtz, Rush Medical College, Chicago, IL; Dr. J. Sogn, NIAID, Bethesda, MD; Dr. K. McAdam, Tufts University, School of Medicine, Boston, MA; Dr. S. S. Mookerjee, Mem. Univ. of Newfoundland, St. Johns, Newfoundland

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INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

1.8

PROFESSIONAL

0.9

OTHER

0.9

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Female protein is a sex limited serum protein of Syrian hamsters and is homologous to human C-reactive protein (CRP) and amyloid P component (AP). CRP and AP are related members of the pentraxin family of proteins, but have separate functional capacities. FP appears to express certain functional attributes of both CRP and AP. Thus, although structurally more similar to AP, FP has been shown to bind PC similar to CRP. Now it is apparent that FP can bind C₁Q and activate complement similar to CRP. On the other hand, FP also has been shown to be a component of hamster amyloid and therefore functionally mimics the AP-amyloid relationship seen in human and experimental amyloid. Furthermore, serum FP and amyloid FP are not sequestered populations but appear to exist in a state of dynamic equilibrium.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00085-07 LPVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders)

Pathogenesis of Aleutian Disease Virus Infection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. E. Bloom

Medical Officer

LPVD, NIAID

Others: A. Cohn

Guest Worker

LPVD, NIAID

R. E. Race

Veterinary Officer

LPVD, NIAID

COOPERATING UNITS (if any)

None

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Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

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INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

1.7

PROFESSIONAL

0.9

OTHER:

0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

The purpose of this project is the study of Aleutian disease of mink (AD), a persistent infection by the Aleutian disease virus (ADV), a nondefective parvovirus. In the past year, we have further compared the infection by ADV in mink and cell culture using Southern blot and dot blot DNA-DNA hybridization analysis. At all levels of infection in cell culture, the predominant form of ADV DNA was the double stranded 4.8 kbp replicative form (RF) monomer, the single stranded virion DNA (SS DNA) was less abundant. In mink tissues, however, RF could be convincingly detected only in spleen and mesenteric lymph node 10 days after infection and the SS DNA was always more abundant than the RF. This suggested that the infection in vivo differs substantially from that in vitro. In addition, 10 day plasma contained large amounts (10^5 genomes/ml) of ADV DNA, probably within virions and some of the ADV DNA detected in highly vascular tissues, like liver, may be diluted by plasma virus. These data suggested that at least spleen and mesenteric lymph nodes are true sites of ADV replication.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00263-03 LPVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Function of the ADV Genome

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

| | | | |
|---------|-------------|-------------------------|-------------|
| PI: | M. E. Bloom | Medical Officer | LPVD, NIAID |
| Others: | C. F. Garon | Research Microbiologist | EM, NIAID |
| | A. Cohn | Guest Worker | LPVD, NIAID |

COOPERATING UNITS (if any)

None

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Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

1.0

PROFESSIONAL

1.0

OTHER

0

CHECK APPROPRIATE BOX(ES)

- | | | |
|---|--|---|
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input checked="" type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors | | |
| <input type="checkbox"/> (a2) Interviews | | |

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The purpose of this project is the study of genome structure and function of the Aleutian disease virus (ADV), a nondefective parvovirus of mink. Further studies on this project have included development for a strategy to molecularly clone genomic segments of ADV field strains not adapted to cell culture. The first strategy was to extract single stranded virion DNA from virus purified from mink organs, to produce duplex monomeric (DM) DNA in vitro via self primed DNA synthesis, and to clone this DNA. To date, we produced this synthetic DM and verified several restriction enzyme recognition sites common to ADV-G and three field strains of ADV (Utah I, Pullman and DK). The second strategy utilized DNA from Hirt supernatants of cells infected with Utah I ADV. Although complete replication of Utah I does not occur in cell culture, conversion of input DNA to replicative form (RF) was sufficient to provide material for cloning. A segment analogous to the EcoRI-Hind III segment of ADV-G (pBM1) was cloned into pUC8. This plasmid, denoted pUT1-1, was found to be indistinguishable from pBM1 by heteroduplex mapping but to have several differences by restriction enzyme mapping. pUT1-1 also directed synthesis of ADV antigens in E. coli similar to those induced by pBM1. Comparison of ADV antigens induced by pBM1 and pUT1-1 is currently under way and has been facilitated by use of a coupled in vitro transcription/translation system.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00380-02 LPVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunological Studies on Aleutian Disease of Mink

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. E. Bloom

Medical Officer

LPVD, NIAID

Others: B. Aasted

Visiting Associate

LPVD, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

PROFESSIONAL

OTHER

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

With the return of Dr. Bent Aasted in August, 1983 to Denmark, this project has been terminated.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00401-01 LPVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Possible Animal Models for the Acquired Immune Deficiency Syndrome (AIDS)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. E. Bloom
R. E. RaceMedical Officer
Veterinary OfficerLPVD, NIAID
LPVD, NIAID

Others: None

COOPERATING UNITS (if any)

None

LAB BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

0.3

PROFESSIONAL

0.3

OTHER

0

CHECK APPROPRIATE BOX(ES)

- ☐
- (a) Human subjects
-
- ☐
- (a1) Minors
-
- ☐
- (a2) Interviews

☐ (b) Human tissues☒ (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is the attempted isolation of a novel infectious agent from patients with the acquired immune deficiency syndrome (AIDS). Two approaches are being taken. The first approach is the inoculation of mink with pooled samples from either terminal AIDS patients or patients with the apparent prodromal phase of AIDS. The rationale is that AIDS patients have a variety of findings similar to ones observed in Aleutian disease of mink, a disorder of immune function caused by a nondefective parvovirus. Some of these findings include plasmacytosis, immune complex disease, glomerulonephritis and hypergammaglobulinemia. The plan is to observe inoculated mink over an extended period of time for development of an AIDS-like syndrome and possibly to assay for evidence of infection with putative AIDS agents such as HTLV III. The second aspect of the study is to inoculate goats and mice with brain material from AIDS patients that have died with the encephalopathy observed in these patients. The purpose for this portion of the work is to determine if a spongiform-like agent (such as scrapie or Creutzfeldt-Jakob) has a role in AIDS.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00086-07 LPVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms of Resistance to Graft Versus Host Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J. E. Portis

Medical Officer

LPVD, NIAID

Others: None

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

2.0

PROFESSIONAL:

1.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

This project focuses on the genetic control of graft versus host disease (GVHD) in a mouse model which in many ways reflects the clinical features of GVHD seen in humans. Bone marrow transplantation has become an increasingly utilized treatment modality for aplastic anemia, congenital immunodeficiency syndromes and several forms of leukemia. GVHD is a major post-transplant clinical problem which appears in unpredictable fashion in recipients of HLA matched MLR nonreactive marrow. Hematopoietic cells from C57BL/6 mice (B6) transferred to unirradiated (B6 X D2)_{F1} recipients induces acute GVHD resulting in pancytopenia, immunosuppression, gastrointestinal lesions and death within several weeks. The existence of an autosomal non-H-2 dominant gene in B6 mice appears to be responsible for this form of GVHD. Confirmation of this hypothesis has come from studies in progeny of [(B6 X D2)_{F1} X D2] X D2 in which induction of acute GVHD segregates in a fashion consistent with one gene. Twenty five recombinant inbred (BXD) mouse strains have been examined, but we were not able to establish linkage of this GVH locus with any known loci in B6 mice. Similar linkage studies are being carried out on an autosomal, non-H-2 recessive gene from D2 mice which when present (homozygous) in the donor of hematopoietic cells induces the production of autoantibody to xenotropic retroviruses in the (B6 X D2)_{F1} recipient. We are currently attempting to determine if this gene is linked to any known locus in D2 mice by examining BXD RI strains. No apparent linkage has yet been established.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00264-03 LPVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Function of Endogenous Retroviruses Expressed During Differentiation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator's Name, title, laboratory, and institute affiliation)

PI: J. L. Portis

Medical Officer

LPVD, NIAID

Others: None

COOPERATING UNITS (if any)

None

LAB. BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

2.0

PROFESSIONAL

1.0

OTHER

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unregulated type. Do not exceed the space provided.)

The possible role of endogenous retroviruses in the maintenance of homeostasis is a controversial subject. The mouse genome contains a profusion of complete and partial integrated proviruses and it is clear that there are mouse strain dependent quantitative and qualitative differences in the expression of these endogenous viruses. Our laboratory is involved in the identification and characterization of endogenous viruses and products therefrom. Our previous identification of an endogenous gp70 expressed during erythroid differentiation in normal mice has provided a framework for further study. We are currently examining in more detail the structural heterogeneity of endogenous viruses expressed in hematopoietic organs. The isolation and characterization of these viruses are in progress and the possible role of these viruses in protection from exogenous infection is being examined. These studies have focussed on the possible role of endogenous viruses in interfering with early events in exogenous virus infection (i.e., binding and penetration). A model has been developed to study these mechanisms using cell fusion as a phenotypic marker of the fusion between viral envelope and cell membrane. This fusion event is a critical step in the process of virus penetration. Cell fusion was found to be pH dependent and independent of an intact viral genome. Using a group of recombinant viruses, this function was mapped to the 5' half of the gp70 gene, a region distinct from that previously considered, based on sequence data, to be responsible for the fusion event [p15(E)]. Further studies should provide information on the site(s) of penetration of murine retroviruses and the relative importance of virus binding and penetration in viral interference.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00199-05 LPVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunobiology of Aleutian Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R. E. Race Veterinary Officer LPVD, NIAID

Others: None

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:

0.7

PROFESSIONAL:

0.5

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to define immune mechanisms and viral characteristics important in the pathogenesis of Aleutian disease (AD). Monoclonal antibodies were used to study antigenic differences among strains of ADV. Antibodies with reactivity for tissue derived Utah I ADV or tissue culture derived ADV-G were clearly delineated. Other strains could be identified by using a large panel of mAbs. New information was obtained regarding the specific antigenic determinants recognized by several mAbs whose precise reactivities were obscure earlier. Thus, some antibodies recognize the major viral structural proteins of ADV-G while others recognize the major viral structural proteins of tissue derived Utah I virus. Still others react with tissue associated low molecular weight proteins probably resulting from *in vivo* proteolysis of larger precursors. Although some of the mAbs had these restricted reactivities, most had broader reactivity for antigen common to some or all of the strains. Selected mAbs were used to study the expression of particular ADV-G virus associated antigens during infection of CRFK cells. Some mAbs recognize predominantly antigens expressed in the cytoplasm of infected cells while others recognize antigens in the nucleus. These mAbs may provide probes allowing us to better understand the kinetics of *in vitro* infection and restrictions some cells are able to exert on viral replication. We also demonstrated viral antigens in the tissues of infected mink using mAbs. Distinct strain specific patterns of fluorescence were observed. If particular viral antigens are especially prone to be involved in immune complex formation and deposition, we hope to identify them *in vivo* using the mAbs that are now available. Such information should clarify our understanding of AD pathogenesis.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00265-03 LPVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Immunobiology of Scrapie Virus Infection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: R. E. Race

Veterinary Officer

LPVD, NIAID

Others: None

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

0.4

PROFESSIONAL:

0.4

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The immediate goal of this project continues to be the establishment of high titered scrapie infected tissue culture cell lines. Scrapie is a naturally occurring spongiform encephalopathy of sheep and goats which causes clinical and pathological changes similar to those of Creutzfeldt-Jakob and Kuru diseases of man. Scrapie grows to high titer in mouse lymphoid tissue and brain but has never been passaged at high titer in any in vitro system. Adaptation of the agent to cell culture will permit detailed characterization of the agent(s) and provide a system for better definition of the pathogenesis of these diseases. We have utilized several approaches designed to provide infected in vitro cell lines. In one approach scrapie infected mice were injected with one of several splenotropic tumor cell lines. Once tumors were established in vivo, they were explanted, maintained in vitro and assayed for the presence of scrapie agent. Eight tumors representing several cell types were used. Results obtained during the past year indicated that no persistently infected cell lines were established using this protocol. A second approach utilized hybridoma technology. Spleen cells from scrapie infected mice were fused with a myeloma cell line. If a scrapie infected cell was a partner in the fusion, persistently infected lines might result. However, no positive cultures were identified. Another approach was begun utilizing a neuroblastoma cell line known to support the replication of at least one isolate of Creutzfeldt-Jakob agent. A second neuroblastoma line known to support the replication of rabies virus was also utilized. We have attempted to infect these cell lines in vitro with purified scrapie material obtained from scrapie infected mice or from scrapie infected hamsters.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00266-03 LPVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic Structure of Murine Retroviruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: L. H. Evans

Senior Staff Fellow

LPVD, NIAID

Others: None

COOPERATING UNITS (if any)

Dr. M. W. Cloyd, Duke University Medical Center, Durham, NC

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:

2.0

PROFESSIONAL:

1.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The major emphasis of this project is the elucidation of the genetic structure of recombinant viruses derived after inoculation of mice with exogenous ecotropic murine leukemia viruses (MuLVs). Such recombinants, termed mink cell focus-forming viruses (MCFs), exhibit an altered host range and are thought to be involved in the pathogenesis of MuLVs. In the initial studies, MCFs derived from NFS mice after inoculation of an erythroleukemia virus (F-MuLV 57) or a lymphocytic leukemia virus (M-MuLV 1387) were examined. It was found that F-MuLV 57 recombined with two distinct endogenous retrovirus sequences and that M-MuLV recombined with a third sequence distinct from the sequence found in recombinants with F-MuLV 57. Further studies indicated that a different strain of F-MuLV (F-MuLV 87) recombines with a sequence which is distinct from the endogenous sequences of F-MuLV 57-derived MCFs but may be identical to sequences found in M-MCFs. These results indicate that a given MuLV preferentially recombines with a specific endogenous retroviral sequence(s) in NFS mice to generate MCFs. One group of MCFs derived from F-MuLV 57 was found to be much more infectious for mink cells than for mouse (SC-1) cells. This group also exhibited an altered Fv-1 tropism (B-tropic) compared to F-MuLV 57 (NB-tropic). All other MCFs in these studies were equally infectious for mink and SC-1 cells and retained the Fv-1 tropism of the ecotropic parent. None of the MCFs derived from F-MuLV 57 or F-MuLV 87 was found to be oncogenic while the M-MCFs induced lymphocytic leukemia in NFS and AKR mice. Examination of MCFs derived from an *in vitro* constructed recombinant between the F-MuLV 57 and the amphotropic virus 4070A indicated that sequences other than the *env* gene determine the specificity of recombination of MuLVs.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00387-01 LPVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders): Phylogenetic Humoral Studies: Antibody Production and Complement Fixation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: H. Etlinger Senior Staff Fellow LPVD, NIAID

Others: J. E. Coe Medical Officer LPVD, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

2.0

PROFESSIONAL

1.0

OTHER

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

The specificity of the antibody response of the mouse was determined by comparing the increase in total immunoglobulin production with immunogen-reactive antibody subsequent to antigenic challenge. Since the increase in both of these constituents was similar, it is concluded that the in situ antibody response is specific. The capacity of hamster female protein (FP) to activate complement was demonstrated. This further establishes the human C-reactive protein/amyloid P component functional chimerism of hamster FP thereby extending the potential biological activities of FP to include enhanced phagocytosis, cytolysis and inflammation.

| | | |
|---|-----------------------------|--|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00386-01 LPVD |
| PERIOD COVERED October 1, 1983 to September 30, 1984 | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Transformation of Hematopoietic Cells by Avian Tumor Viruses | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) | | |
| PI: S. Palmieri Others: None | Staff Fellow | LPVD, NIAID |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Persistent Viral Diseases, Hamilton, MT 59840 | | |
| SECTION | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20205 | | |
| TOTAL MAN-YEARS: 1.0 | PROFESSIONAL: 1.0 | OTHER: 0 |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Two viruses are under investigation: A) the <u>src</u> oncogene-containing Rous Sarcoma Virus (RSV) and B) the <u>myc</u> oncogene-containing Mill Hill 2 virus (MH2). A) Studies during the past year have revealed that RSV has the capacity to transform hematopoietic cells of the erythroid lineage. This effect could be seen both <u>in vitro</u> and <u>in vivo</u>, and the type of transformation observed has also been shown to be distinct from that induced by the <u>erb</u> oncogene-containing avian erythroblastosis virus (AEV) in the following ways: 1) Erythroid cell colonies <u>in vitro</u> induced by RSV have a distinctive morphology, 2) Isolated cell colonies require additional factors for growth in liquid medium, and 3) RSV-transformed cultures contained populations of spontaneously differentiating cells indicating that the cells possess the dual potential to simultaneously self renew and differentiate. Cells transformed by a temperature-sensitive RSV could be induced to differentiate by incubating cultures at the nonpermissive temperature for 4 days. That the pp60src protein is expressed in the cells was demonstrated by a kinase assay. These results indicate that RSV can alter the pattern of erythroid differentiation in a manner similar to, but distinct from AEV. These results also show that the tyrosine-specific kinase encoded by RSV can transform erythroid cells and that the <u>src</u> and <u>erb B</u> proteins may share a similar functional homology, i.e., that the <u>erb B</u> protein also functions as a tyrosine kinase. B) One mutant, which is temperature sensitive for the maintenance of macrophage transformation, has been isolated from a mutagenized stock of MH2 virus. Approximately 400 cell clones were screened during the isolation procedure. Cells shifted to the nonpermissive temperature for 5-7 days differentiated into adherent macrophages in contrast to wild type-transformed cells. The onset of leukemia and death are both delayed relative to wild type MH2 in birds infected with the mutant virus. These findings demonstrate that a virus gene product must be continually expressed for the cells to remain leukemic and that they will undergo a normal-like differentiation once the transforming activity of the virus has been inactivated.</p> | | |

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00267-03 LPVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Endogenous Murine Retroviruses and Leukemogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. W. Cloyd

Senior Staff Fellow

LPVD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

PROFESSIONAL

OTHER

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrebated type. Do not exceed the space provided.)

Due to the departure of Dr. Cloyd to Duke University, Durham, North Carolina, this project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00418-01 LPVD

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunobiology of Equine Infectious Anemia Virus, a Retrovirus Model for AIDS

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

B. Chesebro, Chief, LPVD/NIAID

S. Carpenter, Staff Fellow, LPVD/NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Persistent Viral Diseases, RML, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:

1.1

PROFESSIONAL:

1.1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is the study of the pathogenic mechanisms which occur following infection of horses with equine infectious anemia virus (EIAV), a non-cogenic retrovirus. Particular emphasis is placed on study of hemopoietic cell types infected by the virus as a possible cause of immunosuppression similar to that seen in humans with AIDS. In addition, the role of viral mutation and recombination in generation of viral antigenic variants is being studied with regard to possible mechanisms of viral persistence through avoidance of the specific immune response.



EPIDEMIOLOGY BRANCH
Rocky Mountain Laboratories
Hamilton, Montana
1984 Annual Report
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Annual Report
Epidemiology Branch
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1983, to September 30, 1984

ADMINISTRATIVE REPORT

The Epidemiology Branch, anticipated to become part of the future Laboratory of Molecular Pathobiology (LMP) is currently manned by four Ph.D. scientists plus three supporting staff and one technician (Mr. Jim Whitlock GS-9) who has been assigned to the Operations Branch to be in charge of RML Medium Department and to assist Dr. K. Bergman in the execution of radiation safety procedures. Dr. Willy Burgdorfer, Head of the Arthropod-Borne Diseases Section continued to serve as Acting Chief.

Dr. Jerry M. Keith, Senior Staff Fellow and Ms. Susan Smith, Microbiologist joined EB in January and April 1984, respectively. Dr. Keith is Acting Chief of the Pathobiology Section.

Drs. Aftab Ahmed Ansari and Regina Skelly, both of NAMRU-3, Cairo, Egypt are scheduled to join EB in August 1984 to initiate research in the Immunology Section of the new LMP.

During the year, Mr. Monty Thompson, Histopathology technician resigned to accept appointment at the Penrose Hospital in Colorado Springs, CO. Similarly, Dr. Olivier Péter, a NIH Visiting Fellow since 1982 in Dr. Burgdorfer's Arthropod-Borne Diseases Section terminated his appointment to head the diagnostic department of State Hospitals at Sion, Switzerland.

Under the direction of Dr. Willy Burgdorfer, the EB continued to function as a WHO Collaborating Center for Rickettsial Reference and Research. However, in view of the scheduled absorption of EB by the new LMP, and the drastic changes in research and personnel, the RML will no longer be in a position to fulfill the functions set forth in the contract with WHO. Efforts are under way to assign this responsibility to another rickettsial research institution, possibly the Centers for Disease Control in Atlanta.

Guest workers in Dr. Burgdorfer's Arthropod-Borne Diseases Section included Dr. Linda Logan, USDA, Northeastern Region Plum Island Animal Disease Center, New York; Dr. Birgit Skoldenberg, Danderyd Hospital, Sweden; Dr. Paul Lavoie, M.D., San Francisco; Dr. Robert S. Lane, University of California, Berkeley, California; Dr. Robert Pinger, Ball State University, Muncie, Indiana; and Dr. James A. Swaby, Vector-Borne Disease Unit, USAF School of Aerospace Medicine, Brooks Air Force Base, Texas.

Dr. John Hotchin, M.D., New York State Health Department, Albany, New York was a guest worker in Dr. Hadlow's laboratory.

Annual Report
Epidemiology Branch
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1983, to September 30, 1984

RESEARCH HIGHLIGHTS

The following sections summarize this year's developments in EB's research program. The findings were reported in 22 publications and in an additional 16 papers currently in press.

RICKETTSIOSES

As in previous years, we functioned as a World Health Organization Center for Rickettsial Reference and Research and provided reference agents, guidance and training to investigators and staff of various domestic and foreign health agencies. However, with the EB scheduled to become part of the new Laboratory of Molecular Pathobiology (LMP) and with changes in research and personnel, the functions set forth by WHO no longer can be fulfilled. Efforts are under way to assign the Center's responsibilities to the Rickettsial Research Division at CDC in Atlanta.

Additional investigations of severely ill dogs in southeastern United States strongly suggest the presence of Rickettsia rickettsii physiologically different from those recovered in the Rocky Mountain region. As yet, it is not clear whether the severe clinical manifestations of spotted fever seen in dogs during recent years are related to differences in pathogen-vector-host relationship or are due to genetically derived new strains of R. rickettsii (BURGDORFER, GREENE, PEACOCK).

The rickettsialike symbiotes present in heavy concentrations in the intestinal and genital tissues of ticks belonging to the Ixodes ricinus complex do not interfere with the development of the spotted fever rickettsia, R. rickettsii. Ixodes dammini as well as I. pacificus, experimentally at least, are efficient vectors of R. rickettsii and should be considered potential vectors in nature (BURGDORFER, TODD).

The hemolymph test in conjunction with monoclonal antibodies to R. rickettsii appears to be a highly efficient, dependable, and economical procedure for large scale tick/surveys as well as for the evaluation of ticks removed from a patient (ANACKER, BURGDORFER).

The large outbreak of Q fever in Bagnes (population 4,700), Switzerland where 191 acute cases had been reported, attests to the fact that sporadic outbreaks and epidemics of this disease continue to occur especially in areas where domestic ungulates are raised or animal products are being processed. Because of nonclinical presentation, many outbreaks of Q fever go unrecognized. Two hundred and twenty-four (8%) of 2,962 persons without apparent illness, had antibodies to the causative agent, Coxiella burnetii (PETER, PEACOCK AND BURGDORFER).

Research Highlights (Con't)

A good correlation in serologic titers was obtained in a comparative evaluation between the enzyme-linked immunosorbent assay (ELISA) and the indirect immunofluorescent antibody (IFA) tests of primary, granulomatous, and endocarditis infections of Q fever in man. In sera of Q fever endocarditis patients, the ELISA test detected with sensitivity and reliability high titers of antibodies (THOMAS, PEACOCK, WILLIAMS).

LYME DISEASE

Collaborative studies with several European and American investigators and physicians provided convincing evidence that tick-borne meningoradiculitis (Bannwarth's syndrome) occurring in Europe and in the United States is a clinical expression of the Lyme disease spirochete (BURGDORFER, REIK, TONZ, WEBER AND SKOLDENBERG).

Of 2,483 Ixodes pacificus from California and Oregon, only 22 (1.5%) were found to be infected with spirochetes morphologically and antigenically indistinguishable from the Lyme disease organism isolated from I. dammini. Recovery in BSK medium of spirochetes from a tick collected in Mendocino County, California provides the material for comparative immunochemical studies (BURGDORFER, LANE, GRESBRINK, BARBER).

BORDETELLA PERTUSSIS

In an effort to characterize the biofunctions of the protein subunits of the toxic components of Bordetella pertussis, 24 monoclones recognizing unfractionated pertussis toxin (PT) and 10 monoclones specific for three of the five PT subunits have so far been developed. The monoclones recognize PT in both a natural and denatured state and are extremely useful for screening in the direct-cloning expression system. In addition, two specific peptides from PT were isolated by high pressure liquid chromatography, and their aminoacid sequence of the N-terminus was determined to synthesize specific oligonucleotide probes representing a portion of the toxic gene (KEITH, MUNOZ).

GENESIS OF CHRONIC DISEASE

Because of the long "zero phase" of infection, the question of vertical transmission of scrapie can not be answered now by efforts to isolate virus from fetal, newborn, and even weaned lambs. In efforts to understand the pathogenesis and epidemiology of Aleutian disease in non-Aleutian mink, simple self-limiting infection must be distinguished from progressive disease (HADLOW).

Annual Report
Epidemiology Branch
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1983, to September 30, 1984

HONORS AND AWARDS

The following activities reflect recognition of EB by peers and the scientific community:

Editorial Boards of Journals

Dr. W. Burgdorfer - Acta Tropica: Co-Editor of Current Topics in Pathogen-Vector-Host Research; Journal of Medical Entomology

Dr. W. J. Hadlow - Fundamental and Applied Toxicology

Drs. Burgdorfer and Hadlow reviewed manuscripts for the following journals: Science; J. Med. Entomol.; J. Wildlife Dis.; J. Infect. Dis.; Vet. Pathol.; Infect. Immun.; Acta Tropica; Am. J. Trop. Med. & Hyg.

Professional Posts:

Dr. W. Burgdorfer continued to serve as Acting Chief of EB, and Director of the WHO Reference Center for Rickettsiae and Rickettsial Diseases.

Dr. Hadlow continued to serve on the Advisory Board of the Charles Louis Davis Foundation for the Advancement of Veterinary Pathology; was reappointed to the Committee on Diseases of Sheep and Goats of the U.S. Animal Health Association; continued as Adjunct Professor of Veterinary Pathology, Washington State University; was appointed chairman of the Burroughs Wellcome Fellowship Selection Committee of the American College of Veterinary Pathologists; and was appointed member of the educational committee for the 1984 meeting of the American College of Veterinary Pathologists.

Dr. Jerry M. Keith functioned as jury member for Ph.D. oral defense at Catholique University of Louvaine-la-Neuve, Belgium, February 20, 1984.

Invited Lectures and Participation in Meetings and Symposia

Dr. W. Burgdorfer

- by invitation presented "Discovery of the Lyme disease spirochete" and "The New Zealand White Rabbit - an experimental source for infecting ticks with the Lyme disease spirochete" at the 1st International Symposium on Lyme disease, New Haven, Connecticut, November 16-18, 1983.
- by invitation presented opening lecture "The Vectors and the Etiology of Lyme Arthritis" at the Plenary Session of the 32nd Annual Meeting of the American Society of Tropical Medicine and Hygiene, San Antonio, Texas, December 4-8, 1983.

Honors and Awards (Con't)

- by invitation presented lecture on Lyme disease at the 14th Annual Conference of the Society of Vector Ecologists, San Diego, California, December 14-16, 1983.
- presented at the University of Neuchâtel, Neuchâtel, Switzerland "Current research on Lyme disease and its spirochetal agent," May 19, 1984, and two lectures on "Diseases caused by tick-borne rickettsiae of the spotted fever group" on May 15, 1984.
- by invitation presented "Discovery of the Lyme disease spirochete" at the Max V. Pettenkofer Institute for Hygiene and Microbiology, Munich, West Germany, May 22, 1984.
- by invitation participated as lecturer at the 34th Acarology Summer Program at Ohio State University, Columbus, Ohio, June 24-30, 1984 and presented at the University Hospital lectures on "Ticks and Lyme Disease" and "Spotted Fever: Current Status and Future Trends" and at the Columbus Childrens' Hospital, a lecture on "Lyme disease."
- invited to present a lecture "Zur Entdeckung der Lyme disease (Erythema chronicum migrans) Spirochäte" at the 34th Annual Meeting of the German Dermatology Society, Zürich, Switzerland, May 20-24, 1985.
- by invitation wrote chapters on
 1. Borreliae for the 4th Edition of ASM Manual of Clinical Microbiology.
 2. Vertical Transmission of Spotted Fever and Scrub Typhus Rickettsiae for Current Topics in Pathogen-Vector-Host Research (Praeger Publishers).
 3. The Lyme Disease Spirochete for ASEPSIS - The Infection Control Forum.
 4. Borreliae, Ratbite Fever, Relapsing Fever, and Vincent's Angina for McGraw-Hill's Encyclopedia of Science and Technology.

Dr. W. J. Hadlow

- Participated in the program of the 17th Western Conference of Veterinary Diagnostic Pathologists October 6-7, 1983, at Saskatoon, Canada.
- Attended the 34th Annual Meeting of the American College of Veterinary Pathologists November 29 through December 2, 1983 at San Antonio, Texas.

Dr. J. M. Keith

- by invitation presented lectures at Catholique University of Louvaine-la-Neuve and at the University of Namur, Belgium, February 21-24, 1984.
- by invitation presented seminar in the Human Genetics Department at the University in Ulm, Germany, February 28, 1984.

Honors and Awards (Con't)

Awards

Dr. W. Burgdorfer received a \$5,000 cash award for outstanding service to NIAID and to the field of biomedical research.

| | | | | | | | | | | | | | | |
|---|-----------------------------|--|----------------------|-----------------------------|-----------|--------------|-------------------------|-----------|---------------|----------------|-----------|---------------|-------------------------|-------------|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 AI 00061-22 EB | | | | | | | | | | | | |
| PERIOD COVERED October 1, 1983, to September 30, 1984 | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Natural History of Tick-borne Rickettsiae and Their Public Health Significance | | | | | | | | | | | | | | |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Willy Burgdorfer</td> <td style="width: 33%;">Research Entomologist (Med)</td> <td style="width: 33%;">EB, NIAID</td> </tr> <tr> <td>L. A. Thomas</td> <td>Research Microbiologist</td> <td>EB, NIAID</td> </tr> <tr> <td>M. G. Peacock</td> <td>Microbiologist</td> <td>EB, NIAID</td> </tr> <tr> <td>R. A. Anacker</td> <td>Research Microbiologist</td> <td>LMSF, NIAID</td> </tr> </table> | | | PI: Willy Burgdorfer | Research Entomologist (Med) | EB, NIAID | L. A. Thomas | Research Microbiologist | EB, NIAID | M. G. Peacock | Microbiologist | EB, NIAID | R. A. Anacker | Research Microbiologist | LMSF, NIAID |
| PI: Willy Burgdorfer | Research Entomologist (Med) | EB, NIAID | | | | | | | | | | | | |
| L. A. Thomas | Research Microbiologist | EB, NIAID | | | | | | | | | | | | |
| M. G. Peacock | Microbiologist | EB, NIAID | | | | | | | | | | | | |
| R. A. Anacker | Research Microbiologist | LMSF, NIAID | | | | | | | | | | | | |
| COOPERATING UNITS (if any) Dr. C. Greene, Univ. Georgia, Athens, GA; Dr. B. Klein, Dept. Health and Social Serv., Madison, WI; Dr. O. Peter, Sion, Switzerland. | | | | | | | | | | | | | | |
| LAB/BRANCH Epidemiology Branch (RML), Hamilton, MT 59840 | | | | | | | | | | | | | | |
| SECTION Arthropod-Borne Diseases Section | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20205 | | | | | | | | | | | | | | |
| TOTAL MAN-YEARS: 1.3 | PROFESSIONAL: 0.4 | OTHER: 0.9 | | | | | | | | | | | | |
| CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews | | | | | | | | | | | | | | |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> This project concerns studies of Rocky Mountain spotted fever and other tick-borne rickettsial diseases in the United States and in certain other countries with emphasis on the ecology, identification, and characterization of rickettsiae and their relationship(s) to the respective tick vectors. Source material for experimental comparative studies is obtained through collaboration with outside agencies. The project also considers the cellular and subcellular aspects of interactions between tick-borne rickettsiae and their vectors, particularly the mechanism(s) of interference and the factors responsible for changes in the agent's pathogenicity. Isolation of rickettsiae are made from infected ticks or from bloods of patients or animals by injection into susceptible animals or cell cultures. Characterization of isolates includes serological and biochemical methods (microagglutination, microimmunofluorescence, DNA base composition and protein (SDS-PAGE) determinations). Interactions between rickettsiae and their arthropod vectors is followed by light, fluorescence and electron microscopy of tissues from naturally or experimentally infected ticks. Tissue cultures are being used to study mechanisms of rickettsial development and infection in host cells. The white footed mouse (<u>Peromyscus leucopus</u>) proves to be a useful animal for monitoring spotted fever in nature. Within the distributional areas of <u>D. variabilis</u>, dogs do develop overt clinical spotted fever. Ticks of the <u>I. ricinus</u> complex were shown to be efficient vectors of <u>R. rickettsii</u>. There is no interference between these ticks' symbiotes and spotted fever rickettsiae. Efficiency of the "hemolymph test" greatly improved through the use of conjugates prepared from monoclonal antibodies to <u>R. rickettsii</u>. Immunochemical analyses suggest that the "Swiss agent" - a spotted fever group rickettsia detected in up to 13.5% of <u>I. ricinus</u> from Switzerland - represents a new species of tick-borne rickettsia for which the name <u>Rickettsia helvetica</u> is being proposed. </p> | | | | | | | | | | | | | | |

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00063-14 EB

PERIOD COVERED

October 1, 1983, to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Immune Responses to Rickettsial Infections

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

| | | |
|-------------------|-----------------------------|-------------|
| PI: M. G. Peacock | Microbiologist | EB, NIAID |
| L. A. Thomas | Research Microbiologist | EB, NIAID |
| W. Burgdorfer | Research Entomologist (Med) | EB, NIAID |
| D. W. Hackstadt | Senior Staff Fellow | LMSF, NIAID |
| S. F. Hayes | Biol. Lab. Tech. | OB, NIAID |

COOPERATING UNITS (if any)

Dr. J. C. Williams, Biochemist, USARMIID, Frederick, MD; Dr. O. Peter, Sion, Switzerland; Dr. F. Hayden, Univ. Virginia Med. Center, Charlottesville, VA.

LAB/BRANCH

Epidemiology Branch (RML), Hamilton, MT 59840

SECTION

Epidemiology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

1.6

PROFESSIONAL

1.0

OTHER:

0.6

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to study immune responses in man and animals to natural and experimental rickettsial infections, and to isolate and identify the causative pathogens. For serological investigations, recently developed procedures such as indirect immunofluorescence, microagglutination, and the enzyme-linked immunosorbent assays (ELISA) are being used. It also provides serologic support to other RML units and occasionally also to outside agencies, and includes serodiagnosis of other bacterial or viral diseases under investigation. For the isolation of pathogens, susceptible laboratory animals (meadow voles, guinea pigs, embryonated hen eggs, etc.) and various tissue culture systems (Vero, L cells, etc.) are being used. Serologic tests as well as immunochemical procedures (SDS-PAGE, western blotting) are applied to identification of isolates. Investigations into a disease of suspected rickettsial etiology that killed two persons and hospitalized six others in Virginia, revealed one person to be infected with a typhus agent. Attempts to isolate the organism from brain tissues, blood clots and spinal fluids of several other patients failed. A bacterial agent of hitherto unknown identity was recovered from the brain tissue of a deceased patient. Diagnostic reagents and serologic confirmation was provided in conjunction with a Q fever outbreak in Bagnes, Switzerland where 191 acute cases of this disease had occurred. Of 2,962 patients without apparent illness, 224 (8%) had antibodies to Coxiella burnetii. A comparative study on the sensitivity between ELISA and indirect immunofluorescent antibody tests in the serologic diagnosis of primary, granulomatous, and endocarditis infections of Q fever in man, revealed good correlation. Q fever endocarditis could regularly be identified by high titers of antibodies to phase I particulate antigen. Nonspecific FA staining reactions in sections of human heart valves infected with C. burnetii was found to be eliminated through pepsin and trypsin enzyme treatment of sections prior to FA staining.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00069-23 EB

PERIOD COVERED

October 1, 1983, to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Systematics and Vector Relationships of Certain Parasitic Arthropods

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C. M. Clifford

Scientific Director

EB, NIAID

COOPERATING UNITS (if any)

Drs. H. Hoogstraal, NAMRU-3; D. Sonenshine and P. Homsher, Old Dominion Univ.;
C. Centurion, Univ. of Munich; Miss Jane Walker, Div. of Vet. Services,
Onderstepoort; Mr. Rupert Pegram, Tick Diseases Unit, Lusaka, Zambia.

LAB/BRANCH

Epidemiology Branch (RML), Hamilton, MT 59840

SECTION

Arthropod-Borne Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

PROFESSIONAL

OTHER

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unspaced type. Do not exceed the space provided.)

Dr. Clifford retired. This project has terminated.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00082-23 EB

PERIOD COVERED

October 1, 1983, to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Relation of Viruses to the Genesis of Chronic Disease.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: W. J. Hadlow Research Veterinarian (Pathology) EB, NIAID

COOPERATING UNITS (if any)

Dr. S. B. Prusiner, Department of Neurology, University of California, San Francisco. Dr. D. D. Porter, Department of Pathology, University of California, Los Angeles.

LAB/BRANCH

Epidemiology Branch (RML), Hamilton, MT 59840

SECTION

Histopathology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

1.5

PROFESSIONAL

1.0

OTHER

.05

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

To obtain an insight into the unusual host-virus interactions resulting in slowly evolving diseases, two natural viral infections of domestic animals are studied by simple methods of clinical observation, animal inoculation, serology, virology, and anatomic pathology. These diseases are (1) scrapie of sheep and goats and (2) Aleutian disease of ranch mink. Scrapie is a degenerative disease of the brain caused by an unconventional virus. Replication of the virus in central nervous tissue, which gives rise to the slowly progressive polioencephalopathy, is preceded by many months of replication in extraneural sites, notably lymphoreticular tissues and intestine. Observations on naturally infected lambs and experimentally inoculated fetal and newborn Suffolk sheep provided little information on the early events in the infectious process, especially those events that might bear on modes of natural transmission. This was so because of the long period between exposure to virus and its first detection by mouse inoculation, the only practical way for doing so. The lack of a more suitable detection method and the absence of an immune response to the infection continue to hamper study of this unusual infectious disease. Aleutian disease, caused by a parvovirus, is a chronic renal disease brought about by circulating virus-antibody complexes that become deposited in the glomeruli. Aleutian and non-Aleutian mink were found equally susceptible to infection with several strains of virus. The infection gave rise to viremia in all Aleutian mink but in only some non-Aleutian mink. Disease did not supervene, however, unless the viremia persisted beyond the first few months after exposure to virus. These findings emphasize the need to distinguish between infection and disease when efforts are made to understand the pathogenesis and epidemiology of Aleutian disease. Information obtained from these studies has implications for understanding comparable protracted human diseases.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00268-03 EB

PERIOD COVERED

October 1, 1983, to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Ecology of Lyme disease and related disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | |
|-------------------|-------------------------|-------------|
| PI: W. Burgdorfer | Res. Entomologist (MED) | EB, NIAID |
| A. G. Barbour | Sr. Staff Fellow | LMSF, NIAID |
| S. F. Hayes | Biol. Lab. Tech. | OB, NIAID |

COOPERATING UNITS (if any) Dr. R. S. Lane, Univ. Ca., Berkeley, CA., Dr. M. T. Osterholm, MN Dept. of Health, Minneapolis, MN; Dr. L. Reik, Jr., Univ. CT Health Center, Farmington, CT.; Dr. K. Weber, Munich, W. Germany; Dr. B. Ryberg, Univ. Lund, Sweden; Dr. Tonz, Luzern, Switzerland; Dr. A. Aeschlimann, Univ. Neuchatel,

LAB/BRANCH

Neuchatel, Switzerland.

Epidemiology Branch (RML), Hamilton, MT 59840

SECTION

Arthropod-Borne Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:

1.1

PROFESSIONAL:

0.6

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to determine the natural history of the recently discovered and isolated causative agent of Lyme disease and related disorders. The relationship(s) between the spirochete and its various tick vectors (Ixodes dammini, I. pacificus, I. ricinus, Amblyomma americanum) is being determined by establishing through conventional as well as transmission and scanning electron microscopy (a) the development of the spirochete within the ticks, and (b) the mode(s) of transmission to vertebrate hosts. In cooperation with outside agencies, tick/spirochete surveys are being conducted to determine prevalence of infected ticks in endemic foci. Similarly, the natural source(s) for infecting ticks is being evaluated first serologically (indirect immunofluorescence) and subsequently through recovery of spirochetes from serologically implicated hosts. Of 838 Ixodes pacificus collected in California, 9 (1%) proved infected with spirochetes morphologically and antigenically indistinguishable from the Lyme disease spirochete isolated from I. dammini in eastern U.S. or from I. ricinus from Europe. So far, there is no evidence of spirochetes in the lone star tick, Amblyomma americanum from the TVA Land Between the Lake recreational area. New Zealand white rabbits are susceptible to the Lyme disease spirochete and are useful sources for the experimental infection of ticks with this agent. Of 98 Minnesota patients with tentative diagnosis of Lyme disease, 33 (33%) had IFA titers equal to or greater than 1:64 against a human isolate of the Lyme disease spirochete. Serologic results suggest that tick-borne meningo-radicularitis (Bannwarth's syndrome) is a clinical expression of the Lyme disease spirochete.

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZOI AI 00402-01 EB

PERIOD COVERED

February 1, 1984, to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Cloning and Expression of Bordetella pertussis Toxins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|-----|-------------|-------------------------|-------------|
| PI: | J. M. Keith | Sr. Staff Fellow | EB, NIAID |
| | J. J. Munoz | Research Microbiologist | OD, NIAID |
| | P. Barstad | Sr. Staff Fellow | LMSF, NIAID |
| | L. Mayer | Expert | LMSF, NIAID |
| | S. Smith | Microbiologist | EB, NIAID |

COOPERATING UNITS (if any)

Laboratory of Immunogenetics, NIAID, NIH (J. E. Coligan)

LAB BRANCH

Epidemiology Branch, (RML), Hamilton, MT 59840

SECTION

Pathobiology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

1.25

PROFESSIONAL

.75

OTHER

.50

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (use standard unrefined type. Do not exceed the space provided.)

The project objective is to clone and express selected toxin components from Bordetella pertussis. Of particular interest is pertussis toxin (PT) or "pertussigen" a multifunctional pathogen consisting of a protein complex of 5-6 nonidentical subunits. The biological function of these subunits has not been established. The primary methods employed are oligonucleotide screening of molecular cloned pertussis DNA and direct gene cloning-expression vector system. These two parallel approaches will result in the identification and isolation of the portion of DNA coding for the pertussis toxin. Using these data and DNA clones, genetic manipulation of the pertussis genome may result in a highly immunogenic, nontoxic antigen which could lead to the development of a safer pertussis vaccine. Oligonucleotide Screening. Pertussis toxin was fractionated into subunits using high pressure liquid chromatography. Two of the subunit polypeptides were partially sequenced and, based on this amino acid sequence, two oligonucleotide DNA probes were synthesized. These DNA probes are currently being used to screen fragments of the pertussis genome DNA. Once these fragments are identified, adjacent fragments can be located and sequenced. Computer analysis of the DNA sequence will reveal the molecular structure of the "transcriptional unit" and identification of regulator sites may be possible. These identified fragments will then be used to genetically manipulate the pertussis genome. Direct Cloning-expression System. A cloned library of pertussis DNA fragments will be tested in a direct expression vector system. Antibodies against pertussis toxin will be employed to identify those DNA fragments which produce polypeptides recognized by the antibody. Polyclonal and monoclonal antibodies have been developed in our laboratory which specifically recognize pertussis toxin components in a natural and denatured state; these will be useful in the expression screening. Currently, we are screening the monoclonal antibodies to see what effect they have on the biofunction of the toxin.



ROCKY MOUNTAIN OPERATIONS BRANCH
Rocky Mountain Laboratories
Hamilton, Montana
1984 Annual Report
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Annual Report
Operations Branch
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1983, to September 30, 1984

Introduction

The Branch provides all services necessary to the professional staff in the pursuit of their investigations. This support covers the following areas: procurement, personnel, communications, library services, secretary backup service, grounds care, custodial, security, media preparations, waste disposal, glassware refinishing, photography, animal rearing and care, motor pool, operation of power plants, full maintenance and minor laboratory renovations in every area except electronics.

The Electron Microscopy Section is responsible for research and research collaboration utilizing modern methods of transmission and scanning electron microscopy. Research projects are coordinated with scientists in other laboratories both at RML and Bethesda in order to assure optimal use of microscopy facilities in the study of relevant biological projects in the areas of microbiology, biochemistry, immunology and molecular biology. Members of the Electron Microscopy Section have been involved in the publication of 21 scientific reports during the past year. The EM Section is currently manned by Dr. Claude F. Garon, Head, and three supporting staff.

Dr. William Todd, Senior Staff Fellow in the Electron Microscopy Section completed his fellowship on June 27, 1984.

Guest workers in the Electron Microscopy Section have included: Dr. Richard P. Silver, Office of Biologics, FDA and Dr. Stanley Falkow, Department of Medical Microbiology, Stanford University School of Medicine.

General Overview of the Responsibilities of Operations Branch

The fiscal and procurement department manages a budget of \$1,217,000. Payroll is not included in this figure. It covers only the purchase of supplies and minor equipment used in the operation of the laboratories. Timekeeping and the correction of errors in the payroll are also handled in the unit.

Personnel handles all actions and advises on personnel matters. This department also is charged with the operation of the Comprehensive Employment Training Act (CETA) in association with the local Montana State Employment Office. Through the year, we have averaged two persons on this program, serving a two-month appointment. The maximum time a person may spend on the program is two months. Hence, we are constantly interviewing and employing people under the program. Also handled by Personnel are persons under the following programs: Stay-in-School, Work Study, Government Summer Program, Visiting Program, and students studying for advanced degrees.

Custodial services are provided in the five laboratory buildings daily. Security is provided in the form of a guard on duty every night of the year.

Most of the media used in the research laboratories is prepared in a special laboratory by a technician. All glassware is cleaned and sterilized in the glassware department for reuse in the laboratories.

The Graphic Arts Department provides full professional services necessary in the laboratories with the exception of medical artistry.

The Animal Unit raises 12 strains of mice, 8 strains of hamsters, and 1 colony of microtus. They breed and raise approximately 90,000 animals a year. An additional 7,000 animals are purchased annually from outside sources, including mink, sheep, rabbits, mice, and hamsters. After rearing, care is provided for these animals while they are under experiment.

The maintenance department, through the power plant, provides heat, steam, air and vacuum to the laboratories. Also provided are air conditioning, compressed air, and demineralized and distilled water. A motor pool consisting of 10 vehicles is maintained. Grounds care, including snow removal, is provided.

With the exception of the electronics work, all maintenance is done by the staff. This includes plumbing, electrical, sheet metal, carpentry, and refrigeration, including ultra low temperature boxes.

Labor management work is handled by the Chief of the Branch.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00201-05 RMOB

PERIOD COVERED

October 1, 1983, to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structural Characterization of Integrated Viral Genomes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Claude F. Garon, Section Head, RMOB/NIAID

COOPERATING UNITS (if any)

Takis S. Papas, LMO/NCI; Richard P. Silver, FDA; Malcolm A. Martin, LMM/NIAID;
Steven E. Straus LCI/NIAID

LAB/BRANCH

Rocky Mountain Operations Branch (RML), Hamilton, MT 59840

SECTION

Electron Microscopy

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

1.5

PROFESSIONAL

0.8

OTHER

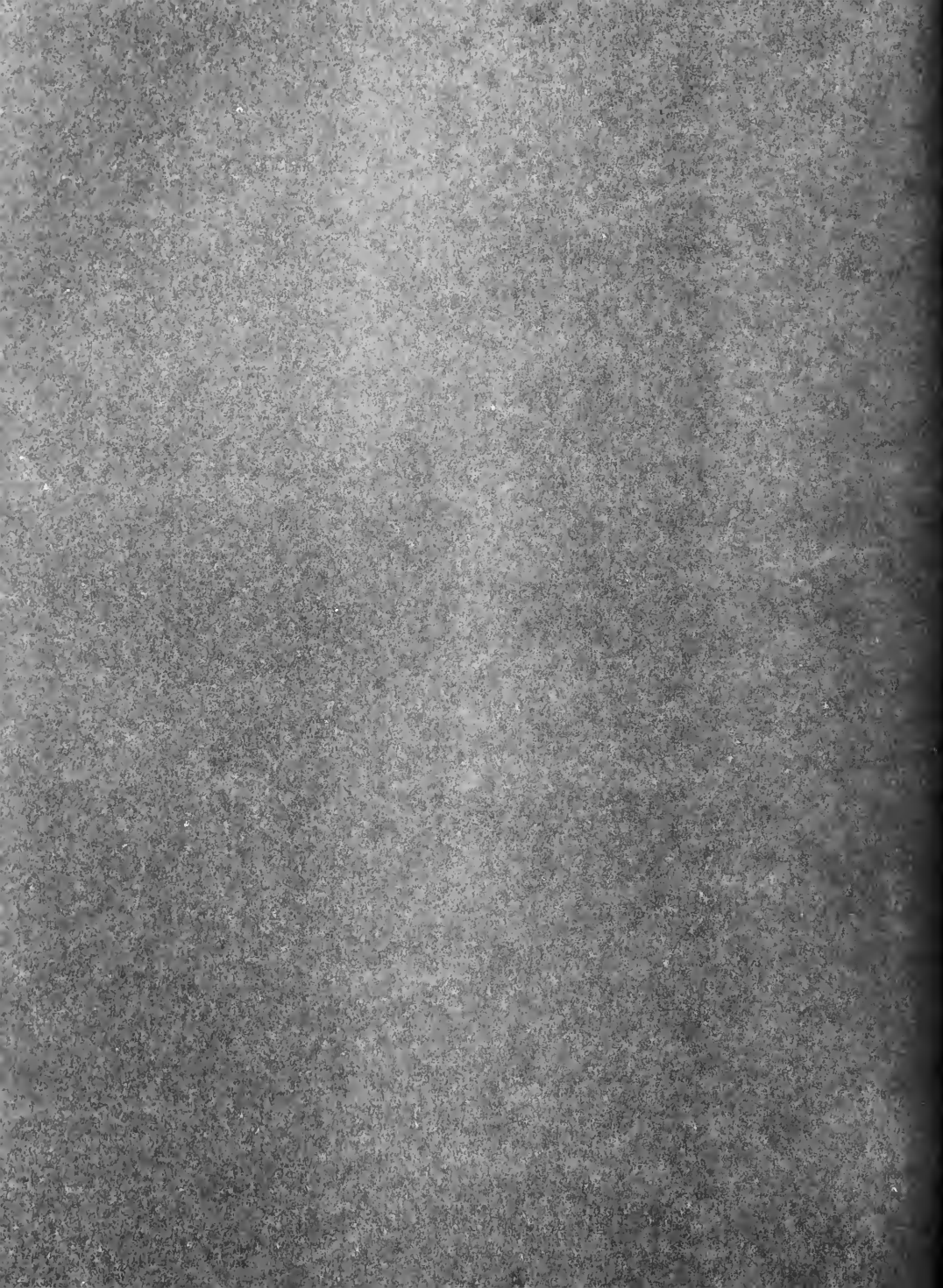
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☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

While several classes of viruses form stable associations with their hosts by integrating one or more copies of their genomes into the host cell DNA, retroviruses provide a unique and important system for the study of integrative recombination. Retroviral genomes are integrated with high efficiency at specific sites within the viral genome, but at a large number of sites in the host chromosome. Often a consequence of this integration event is a readily detectable change in cell growth. Modern methods of molecular cloning and analysis allow for the detection and amplification of rare DNA sequences such as an integrated viral segment. Molecular clones of several newly integrated retroviral genomes were produced in either plasmid or bacteriophage cloning vehicles using recombinant DNA techniques and were characterized using electron microscope heteroduplex and R-loop methods. Detection of sequence homology even when interrupted by intervening cellular DNA is often accurately mappable in the electron microscope using these methods. These studies have not only shown the arrangement of integrated viral sequences within infected host cell DNA; but have also demonstrated the presence and sequence arrangement of certain viral transforming sequences within normal, uninfected host cells as well. The major objective of these studies has been the application of physical and biochemical techniques to assess the influence of integrative position or flanking cellular sequences on subsequent viral function and to define in molecular terms those events which take place during integrative recombination.



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Bethesda, Md.



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